

Too much panic over AIDS?

Most governments have reacted somehow to the AIDS epidemic, some regrettably illiberally but none has given epidemiology its due, in particular its capacity to rid us of uncertainties.

THE British government, as if to deny the reputation of the British for understatement, has creditably done more than any other to take at their face value the pleas of knowledgeable people, last year's committee of the US national academies of science and of engineering, for example, that the first defence against AIDS should be public education. It has acted quickly and even radically, overturning conventions on the way.

The consequences have been twofold. First, British television programmes appear now to consist largely of solemn discussions of the mechanics of AIDS infection, which are complicated, and of the means of avoiding it, which are rudimentary but no more certain on that account. From a state of affairs in which, a few months ago, the word 'condom' was not useable in polite society, Britain has slipped effortlessly into a condition in which the public and commercial TV broadcasting organizations have cooperated in a demonstration (*inter alia*) of how don one of those disdained devices. Publications of all kinds appear to have risen to the challenge of saying what AIDS is all about, perhaps welcoming this implicit licence to tell something like it is. The second consequence should have been predicted; not merely are some people offended by the language of the message, but others are inclined to dispute its necessity. Newspapers tell true stories of physicians besieged by telephone enquirers. Others say that there is too much "panic" about AIDS. The origin of this sceptical reaction is merely that the British government, for all its willingness to spend money on public education, and for all the grave statements of public spokesmen that AIDS is a sombre threat to individual survival, has not said openly why it has (uncharacteristically) sanctioned all this spending: it believes (rightly) that it is squarely within the bounds of possibility that AIDS is a threat to the survival of whole populations, not just of individuals.

In cruel politics, it may be asking to much to ask that a government should be that explicit. It may be prudent to base policy on worst cases, but imprudent to say so in case the worst case does not materialize. If, in due course, it turns out that the transmission of the AIDS virus between heterosexuals is not facile enough to sustain the present epidemic, there will be many who will say, even on present evidence, that the British government has panicked needlessly, and should not therefore be trusted with other public duties, the defence of what used to be called the realm among them. It may therefore help the cause of rationality in what is necessarily a complicated matter that those concerned should come to grips with articles like that from Robert M. May and Roy M. Anderson on page 137. That is a technical account of what might theoretically be expected of the course of a sexually transmitted disease, which AIDS is, but all the more important for that reason.

Promiscuity

The chief inference from May and Anderson must be that, so far, little is known of the natural history of AIDS. For example, the incubation period, or the interval between infection and the appearance of symptoms of disease, appears at present to be 4 to 5 years, but that is chiefly because there has been no more time than that for the consequences of the infection to become appa-

rent. Five years from now, the same authors may be saying that the incubation period, on the evidence then available, is 9 to 10 years (which might imply that those infected would have had twice as long to pass on the infection to others). Similarly, as May and Anderson make plain, there is no sure way of assessing the chance that an infected person will infect another as a consequence of a single act of sexual intercourse. The best they can do is to calculate the product of that probability with an arithmetical function which is essentially a measure of the contribution of promiscuity to the transmission of the AIDS virus.

Carriers

The conclusion is simple enough — promiscuous individuals are doubly ill-starred, because they are more likely to be carriers and also more likely to encounter carriers, so that their influence in the propagation of the epidemic is proportional to the square of the number of their sexual partners. All that is easily accepted, but has little bearing on the estimation of the crucial parameters — the incubation period, the duration of infectiousness of infected people, its distribution in time (episodic or otherwise?) and the likelihood of transmission (per encounter) between people of different sexes in different kinds of sexual encounters. In time, it could be that a better knowledge and understanding of the occurrence of AIDS in Africa could help pin down these crucial numbers. But that will not be easy, given the unwillingness of African governments to acknowledge that they (and we) have a problem to confront. The chance that useful epidemiological data might be gathered from Haiti, supposed a few years ago to be the staging post between Africa and the United States, is similarly slim.

What, in these circumstances, should prudent governments attempt to do? (The word 'prudent' must be read both ways, as a means of safeguarding constituents and as a device for avoiding as much as possible of the political trouble that attends messengers carrying bad news that turns out to be wrong.) Surely the answer must be that epidemiology has a great deal to learn, and then to teach, about the nature and the dynamics of the present epidemic? The mindful British government which, earlier this month, endowed the Medical Research Council with the best part of £20 million to find a vaccine prophylactic, should now get out its bigger cheque-book to take on the more expensive, if less dramatic, work of understanding the mechanism by which AIDS is transmitted. How did so-and-so catch it, from whom, by what route, where did that infection come from and how common in the British population are the practices involved? Undertakings such as these are expensive because they entail talking to many more people than would be employed even on the most ambitious research programmes.

What goes for the British government applies to others. Unfortunately, most politicians appear to have settled for the quick fix, technological or otherwise. Both in India and in Belgium, it is now the practice that foreign students must be tested for AIDS on arrival. The information will of course be meaningless in respect of those who are infected, but in whom the process of sero-conversion (the appearance of antibodies) has not yet occurred; no doubt, the practice of this kind of ritual screening



will persuade constituents that something is being done. Elsewhere, resources are being poured in two directions — the search for prophylaxis and, more immediately (and beneficently), the care of the sick. Nowhere is enough attention being paid to the need for an understanding and a quantitative description of the mechanism of this infection. The temptation to neglect this kind of work is understandable; epidemiology is slow, even slower than finding a vaccine. But on the assumption that AIDS will cast a shadow over at least several decades to come, is that not a case for starting soon? On the assumption that AIDS has presented us all with an unprecedented threat, to which countermeasures may not exist, may not the only visible remedy for a sense of panic be an understanding of the disease? □

Academic-wall writing

Discrimination between universities is beginning to bite in Britain. Where will it lead?

WITH the passage of time, the future pattern of British academic life becomes clearer, but not much brighter. Universities individually are beginning to come to grips with this year's allotment letters from the University Grants Committee (UGC), of which there is one universally good thing to be said this year — that universities will know where they stand in good time, and sufficiently far ahead of the financial academic year beginning on 1 August for them to hope to accommodate themselves to the changes under way. That, at least, is something to be grateful for. But the principal change now under way will please only some of UGC's constituents, those that have done relatively well in the system-wide competition for attention for having done well at research.

A year ago, in planning for the present academic year, UGC carried out a hasty assessment of the virtues of the separate institutions and shared out the funds at its disposal with the proviso that no single university's position would decline relative to the average decline by more than 1.5 per cent. The same "safety net", as the standard euphemism describes it, is also in operation this year. But the universities, many of whose employees are numerate, have used the interval to calculate that a 1.5 per cent relative cut, accumulated over five years, would amount to a disadvantage compared with the average of nearly 10 per cent. Because the bulk of university spending is on salaries and related benefits, it has suddenly become apparent to university administrators and even to their academic employees that it will not be long before there is a sharp division between some sheep and some goats; the establishments in between, whose nature has not yet been determined or clearly signalled, will be a dwindling group.

The way this year's cookie has crumbled is not very different from what happened a year ago. By no standards has any university been treated really generously; indeed, the funds available to UGC have increased by only 3.3 per cent in a year when the retail price index is likely to have done more damage than that to institutions' well-being, while most institutions are still at a loss to know how they will meet the increased pay bills due from last April. It is interesting that the places winning larger shares of the cake this year are, like last year's winners, some but not all of the newer creations — universities such as those of Bath, Warwick and York (with increases of recurrent grant of more than 6 per cent). Some (but again not all) of the large city universities have, like Oxbridge, also done better than the average, if modestly. At the other end of the spectrum are such places as different as the Manchester Business School and the University of East Anglia, which have done no better than the bare minimum. The Celtic fringe in Wales and Scotland has again come poorly out of the settlement, but piquantly, Heriot-Watt university, until recently a kind of polytechnic, has done better than its immediate neighbour Edinburgh (with increases of 4.3 per cent and 1.3 per cent

respectively). The University of Wales Institute of Science and Technology, with 3.5 per cent, is better off than its older neighbour, the University College of Cardiff, with which it is in a prolonged merger dispute. There is plenty in all this for common-room heads to cluck about.

What cannot be dodged is the trend. Some institutions are on the way down and others may be staying afloat, or even marginally improving their positions. Moreover, as academic governing bodies throughout Britain know too well, even marginal changes can hugely determine their freedom of manoeuvre. A university losing real income may have to contemplate getting rid of people; one whose income rises will have the luxury of being able to employ young people with new ideas. So those that have done well in this year's competition may expect to do even better in future years. The Peter Principle ("To him who hath, etc...") applies. At this rate, it may not be very long before some British universities have become teaching institutions, of the kind that Mr Kenneth Baker, the Secretary of State for education and Science, was brooding about last week (see page 116). What remains to be determined is how the weakening institutions will respond to the adversity that afflicts them.

The first and natural reaction of such a place, and of those who work in it, is to protest that the process of enforced decline is unfair. So, in many senses, it is. There are, for example, well-attested anomalies in the way in which the funds have been shared. Many zealous academics have shown that UGC's ratings of departments accord with no known objective measure of their productivity in research. A more serious objection to the way that things have been done may be the tendency to take at their face value universities' own estimates of the cost of teaching their students. Small departments (and, therefore, small universities) get the short straws. Too little account may have been taken of industrial contracts, as distinct from public research grants, in assessing the non-teaching functions of universities. And it is a plain fact that even the least successful of the British universities competing for next year's funds are well supplied with creative and distinguished scholars who cry that insufficient account is taken of their contribution to the distinction of their institutions. The proper rejoinder has two parts. First, restrained commiseration; it may be bad luck to find oneself in a sinking ship, but is that necessarily a circumstance for which the occupants have no responsibility? Second, so what? Even if a substantial part of the British university system may in future have no great involvement in research, that does not mean that enterprising academics need be denied opportunities for scholarship. And who says that the education of the young can be left to the second-rate?

The plain truth is that the reshaping of the British university system now under way is necessary not only for reasons of economy but of scholarship as well. Too many parts of the system are too thinly supplied with talent to be creative on their own. It is more than twenty years since the Science Research Council, the predecessor of what is now the Science and Engineering Research Council, announced its firm intention to concentrate resources. Even earlier, UGC had made a few abortive attempts in that direction. But nothing much happened. The pity now is that the change has been enforced not by reason but by several years of public parsimony whose most conspicuous effects have been uneconomic savings, a waste of too many people and a general sense among academics that nothing is ever for the best. The research enterprise has been so damaged in this process that people have forgotten that what the system needs most of all is a mere handful of demonstrations that excellence still survives. Even the less lucky universities have a long-term interest in that state of affairs, for how else can their members and future students be persuaded to aspire to intellectual goals? Those which have been lucky in the competition, and others, have a duty to demand that the more diverse system now in prospect provides opportunities for universities which may not be distinguished in research to improve.

Franco – US agreement on AIDS test within sight

- AIDS patent dispute near end?
- France and United States call truce

Washington

THE tangled legal wranglings between the US Department of Health and Human Services (HHS) and the French Institut Pasteur over the discovery of the virus that causes AIDS (acquired immune deficiency syndrome) and over the patent for a blood test that resulted from the discovery may be coming to an end.

A joint statement by Institut Pasteur and HHS released last week heaps glowing praise on both Robert Gallo of HHS's National Institutes of Health (NIH) and Luc Montagnier of the Institut Pasteur for their work on AIDS. But it then takes the curious tack of decrying "sensationalistic journalism" and condemning the "inaccuracies that have appeared recently and in the past describing the dispute between the two parties". The statement goes on to suggest that the legal dispute "involves highly technical issues of patent law" and does not diminish the contributions of Gallo and Montagnier.

The legal dispute concerns a patent awarded to Gallo for an antibody blood test for the AIDS virus. The Institut Pasteur has claimed that Montagnier should have received the blood test patent, as he had submitted a patent claim to the US Patent and Trademark Office some seven months before Gallo. The Patent Office has initiated an investigation that could lead to the patent reverting to Institut Pasteur.

Another dispute between the two institutions involves the sample of the AIDS virus that Montagnier gave to Gallo in 1983 for his research purposes only. The Institut Pasteur claims that Gallo used the French virus isolate to perfect his blood test, violating his agreement not to use the material for commercial purposes.

Both sides in the dispute are being extremely cautious in their public statements. Chuck Kline, a spokesman for HHS, says the language of the statement was only arrived at after multiple discussions between both sides' lawyers.

The joint statement takes pains to smooth over differences between the NIH and Institut Pasteur. It was issued by James Wyngaarden, director of NIH, and Raymond Dedonder, director of the Institut Pasteur and their respective lawyers.

The statement also appears to be the harbinger of an agreement, proclaiming that negotiations will lead to an "amicable settlement which will be honourable for all". A final line states that a settlement

"would recognize the important contributions of Dr Gallo and his colleagues and Dr Montagnier and his colleagues leading to our understanding of AIDS and its diagnosis, and such a settlement should in no way be interpreted as providing either party with an advantage over the other party".

Both sides appear anxious to reach a settlement by the end of the month when French Prime Minister Jacques Chirac visits Washington. The settlement will probably include some mechanism for sharing financial benefits from patented blood tests, possibly through an international foundation first proposed by the United States last May (see *Nature* 321,185;1986).



Montagnier (left) and Gallo. On the way to agreement?

Offending articles had made claims that "in the war against AIDS, scientific truth was among the first casualties" and suggested that Gallo clung to the idea that the AIDS virus — what Gallo then called HTLV-III and Montagnier called LAV — was more closely related to HTLV-I and II than it actually is. By doing so, Gallo indirectly caused many scientists to go off on the wrong track, the report claimed. Gallo had earlier discovered HTLV-I and II, two human retroviruses that apparently cause cancer.

The reports of Gallo's and Montagnier's work examined the similarities between the viral isolates used by them in developing their blood tests. Restriction maps have shown these two viruses to be indistinguishable, but sequence data show there are differences. Montagnier had given a sample of his virus to Gallo in September 1983, around the time Gallo says he succeeded in growing his own virus.

Last week Gallo and Mikulas Popovic were granted a patent for growing the AIDS virus in several different cell lines. This new patent covers virtually all cell lines in which the AIDS virus is currently grown.

Joseph Palca

Supreme Court reverses AIDS judgement

Washington

THE US Supreme Court, rejecting the argument of the Reagan administration, has ruled that a federal antidiscrimination law protecting handicapped workers extends to people with AIDS (acquired immune deficiency syndrome) and other contagious diseases. According to the Justice Department, an employer's fear of contagion, whether reasonable or not, was sufficient reason for firing Gene Arline, a Florida schoolteacher discharged from her job because she had tuberculosis. In a 7-2 decision, the court rejected this argument, ruling that employers receiving federal funds cannot deny jobs to people with contagious disease simply because of their own perceptions about the illness. Justice William Brennan, who wrote the opinion for the court, said "it would be unfair to allow an employer to seize upon the distinction between the effects of a disease on others and the effects of a disease on a patient and use that distinction to justify discriminatory treatment".

Reaction from the administration was muted. "The decision speaks for itself... we said what we had to say in our amicus [advice]", stated Justice Department spokeswoman Deborah Burston-Wade.

The ruling coincides with increasingly heated controversy over the rights of AIDS victims. One side argues that critical public health measures have been blocked by oversensitivity to civil liberties; the other side rejoins that protection of those liberties is the only way to get the cooperation needed to stem the AIDS epidemic.

Representative William Dannemeyer (Republican; California) attacked the public health community at a symposium on AIDS and employment held the day after the Arline decision. "Better that some people die of AIDS than that we transgress a group's civil rights", was his version of the US public health AIDS strategy. He claims that the male homosexual lobby is blocking measures such as mandatory blood tests and reporting names to the Public Health Service of those with positive tests.

Nan Hunter, of the American Civil Liberties Union (ACLU), argues that fear of discrimination and loss of rights is crippling the nation's response to AIDS. Cooperation in voluntary testing and safe sexual behaviour is essential, she says, but why be tested if it means losing fundamental rights? The objection is not to reporting numbers, Hunter says, but to the collection by the government of names and other information that can be used to discriminate against people in jobs, housing and medical insurance.

Nancy Heneson

Baker urges concentration of research in fewer universities

London

Mr Kenneth Baker, the British Secretary of State for Education and Science, while still preoccupied with his running battle with schoolteachers over pay, is planning also to turn his attention to the administration of those parts of the research enterprise within his charge — academic research and the institutes of the four science-based research councils.

Further reorganization of the research council system is, however, unlikely. Mr Baker seems content with the present division of responsibility between the research councils, but is anxious that their work should be more pointed. One focus of concern is the extent to which research council funds are committed in advance by long-term obligations.

Mr Baker is less certain of the constitution of the Advisory Board for the Research Councils (ABRC), which gives him advice on the partition of the annual science budget among the research councils. One possibility is that the most interested parties, the research councils themselves, might not be directly represented on the council. Another is that the ABRC may be better provided with people able to monitor changing patterns of research at British institutions. Much will no doubt depend on the views of the ABRC chairman Sir David Phillips, of whom Mr Baker seems thoroughly to approve.

On the university front, the drive towards the concentration of research in a smaller number of universities or university departments, which has already influenced the allocations of funds to individual universities in the present academic

tions and, particularly, by better salaries. In the long run, he believes, the long-standing annual loss of something like 1,000 people a year, which may recently have increased, may require that attention should be given to radical changes in British tax arrangements to make them more competitive with those now in force in the United States under the provisions of last year's Tax Reform Act.

In the world of practical politics, however, Mr Baker says he will be looking at a variety of other measures. He hopes that the Royal Society's study of the loss of skilled people from Britain, due to be completed soon, will have some practical suggestions to make. He also wonders whether it may be necessary for some employers to take a leaf out of another US book, and to look to countries such as India for replacements for lost British academics.

John Maddox

New civil service pay deal betters par

London

HIGH fliers in the British scientific civil service specializing in disciplines much in demand will reap substantial financial rewards in the next few years from the introduction of a radical new pay structure. As part of an offer by the British Treasury to the Institution of Professional Civil Servants (IPCS), there will be guaranteed increases of pay for all staff in the next two years, with the promise of further increases for people in fields with "recruitment and retention problems".

The pay offer covers IPCS staff working in the public service, not only in the civil service. IPCS is the negotiating body for some 60,000 scientists and technologists, many working for quasi-autonomous organizations such as the UK Atomic Energy Authority.

Those concerned will receive a pay increase on 1 April amounting to the percentage increase of the Retail Price Index in the year to the end of last month, expected to be about 3.9 per cent. There will be further pay increases in September and next April making a total estimated at 15 per cent. The new merit increases will also be effective from September.

One striking feature of the pay award is that it appears to breach the government's position that pay increases in the public sector should not exceed the rate of inflation. But the new structure has also been designed to reward individual performance and in such a way that merit awards are not uniformly spread over large diverse groups of scientific workers.

According to the Treasury, the intention is that the rewards of members of the scientific civil service should be determined by the performance of small, not large, groups in which they work and even on an individual basis, taking account of the skills employed as well as of "conditions in different parts of the country". The last of these provisions is in line with the British government's wish, put forcefully to a meeting of the National Economic Development Council last week, that pay bargaining should take account of the variation of living costs between different parts of Britain.

The scheme proposed for the scientific civil service aims to reward personal performance in ways that match those obtainable in industry. An essential part of the proposals is that there should be more effective individual job evaluation. The proposed deal, which will be effective until August 1989, will be discussed at a special conference of IPCS on 8 April.

Bill Johnstone

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS



Kenneth Baker sees tax reforms as a cure for the 'brain drain'.

year and, in a letter to universities last month, for the academic year beginning in August, will be encouraged. Last week, Mr Baker was even wondering aloud whether "some universities should become teaching institutions", with no substantial research activity.

Meanwhile, Mr Baker acknowledges that something must be done about the rate at which British scientists are being tempted abroad by better working condi-

Tokyo

ONE of the first images taken by Japan's marine observation satellite Momo, launched on 19 February. This non-corrected false-colour image, taken by the satellite's multi-spectral electronic self-scanning radiometer on 23 February at about 10.50 a.m., is of Ohmura Bay off Kyushu (Nagasaki airport is visible in the bay linked to the mainland by a causeway). Blue and green represent bands 1 and 2 in the visible spectrum (0.51 – 0.59 μm and 0.61 – 0.69 μm , respectively), and red is band 4 in the near infrared (0.72 – 0.80 μm). The photograph spans about 20 km east to west and 36 km north to south. □

NASDA

Japan's unpopular mental health laws to be revised at last

Tokyo

JAPAN's mental health laws are about to undergo their first revision in 22 years. The reforms, contained in a bill to be submitted to the Diet by mid-March, are a response to severe international criticism of Japan's mental health system.

The sorry state of Japan's mental hospitals first caught worldwide attention in 1984 when a scandal erupted at Utsunomiya Hospital. It was reported in the press that violence and confinement were regularly used to subdue patients, patients were forced to labour at a deep-freeze food factory owned by the hospital superintendent's family, others were made to act as assistant nurses and give injections to fellow patients. And, worst of all, two patients were allegedly beaten to death.

Representatives of the International Commission of Jurists (ICJ) and the International Commission of Health Professionals (ICHHP) visited Japan on a fact-finding mission in May 1985 at the invitation of lawyer Etsuro Totsuka, one of Japan's foremost campaigners for mental health reform. A 94-page report released by the mission in September last year concluded that "the present structure and function of Japanese mental health services create conditions which are conducive to inappropriate forms of care and serious human rights violations". The major areas of concern identified by the mission were a lack of legal protection for patients, and a system of care characterized by a preponderance of long-term institutional treatment with little community treatment or rehabilitation. The commission recommended that an independent body be set up to review involuntary hospitalizations.

While the numbers of mental inpatients in countries such as Britain and the United States have been falling, Japan's inpatient population has grown more than twentyfold over the past 30 years to 340,000, about 80 per cent of them involuntary admissions. Most of those confined against their will were committed by "consent" (*doinyuin*) under section 33 of the present mental health act. Consent is obtained not from the patient but from the family or a legal guardian. As there is still a considerable social stigma associated with mental illness in Japan, families are often only too willing to commit their mentally ill relatives. The only medical opinion required for such confinement is that of the superintendent of the hospital.

The main revisions to the law instituted by the Health and Welfare Ministry are: voluntary admissions will be encouraged but all of the clauses for compulsory admission will be retained in modified form (under the old law there was no

clause for voluntary admission); prefectural review councils will be set up to hear patient's complaints and to screen involuntary admissions; local bodies and welfare organizations are urged to establish rehabilitation centres.

Etsuro Totsuka welcomes the revisions but still sees serious defects. "Consent hospitalization" is retained under a new name "protective hospitalization", the only change being that the medical opinion of a "designated doctor" rather than the hospital superintendent must be sought before committal and the patient's case will be periodically reviewed by the prefectural review council. But ministry officials expect that the designated doctor will come from the hospital in which the patient is to be hospitalized. Totsuka also questions the independence of a prefectural review council of three designated doctors, one lawyer, and one "man of knowledge and experience".

Professor Haruo Akimoto, former director of Tokyo Metropolitan Matsuzawa Hospital in Japan, also expresses doubts about the independence of the review council and he advocates inclusion of lay organizations such as the National Federation of Families of the Mentally Ill in Japan; Totsuka favours the British system of Mental Health Review Tribunals which consist of one doctor, one lawyer and one lay person.

A third serious defect is the lack of an equality clause to eliminate discrimination against the mentally handicapped. At present mental patients in Japan are barred from entering art museums or swimming pools; they cannot take up certain professions, such as hairdressing; and general rehabilitation and welfare services are not available to psychiatric patients. The Health and Welfare Ministry has asked the various ministries concerned to eliminate such discrimination, but no legislative action has been taken.

Finally, although there is a clause in the law calling for free, uncensored communication by patients with the outside world by letter, telephones are not mentioned. And there are no provisions in the law for meeting lawyers or independent doctors.

Both Totsuka and Akimoto welcome the clause calling for improved rehabilitation services. But they question whether the words will be backed up with government money. Akimoto hopes that "the new law is not just a gesture by the government intended to appease international criticism but that it will really assist rehabilitation and independence of the mentally ill". What is needed, he says, is reform of the hospitals, not just reform of the law.

David Swinbanks

NASA science head

Washington

LENNARD Fisk has been named to succeed Burton Edelson as head of the office of space science and applications at the National Aeronautics and Space Administration. Fisk is at present a professor of physics at the University of New Hampshire in Durham. Until his appointment was announced last month, he was chairman of the space science working group of the American Association of Universities and chairman of the National Academy of Sciences committee on solar and space physics.

Fisk's selection has been warmly received in the academic community. Thomas Donahue, chairman of the National Academy of Sciences space science board, calls Fisk "a card-carrying scientist" who will be able to work well with the space science community. His appointment takes effect on 6 April. J.P.

Death of Steve Prentis

London

STEVE Prentis, who died in a car crash on 27 February, had only recently become director of the Banbury Center and director of publications at the Cold Spring Harbor Laboratory, Long Island, where he was to be the editor of the journal *Genes and Development*, which was launched last week. Before moving to the United States, he had been based at the Cambridge offices of Elsevier in the United Kingdom, where he was initially editor of *Trends in Biochemical Sciences*, and then founding editor in turn of *Trends in Biotechnology* and *Trends in Genetics*. A second edition of his widely praised book, *Biotechnology: A New Industrial Revolution* has just been published. P.N.

Plea for return of exiles

London

THE University of Kabul has issued an appeal to former employees who have fled from Afghanistan to return home. Their jobs are still open, the appeal said, their reputations are still held in respect, the laboratories and libraries are awaiting them. The university will put at their disposal "every available opportunity" to prepare themselves for their return to teaching and to help them to carry out "better research".

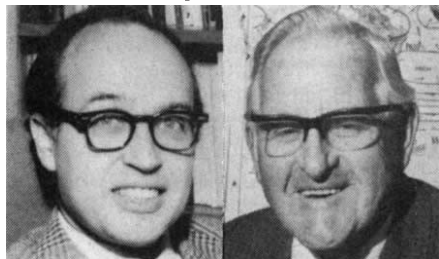
The appeal, broadcast by Kabul radio, was presented as part of the current policy of national reconciliation. But the university also confessed its need of the émigrés. The lecturers now working there do not, it said, have the "knowledge, experience and energy" of those who went abroad. In fact, according to the Refugee Studies Programme of the University of Oxford, very few Afghan émigré academics have managed to find professional posts abroad, and most are still living in refugee camps. V.R.

Faisal international prizes in search of winners

Riyadh

THE short and somewhat shaky history of the King Faisal International Prizes was continued this week with the 1987 award ceremony in the Saudi-Arabian capital. Of the five annual prizes of £60,000 each, two of the three in Islamic/Arabic studies were not awarded for lack of nominations of sufficient quality. But there is no disputing the credentials of this year's winners of the medicine and science prizes, New Zealander Professor Barrie Jones of the International Centre for Eye Health at Moorfields Hospital in London and Sir Michael Atiyah, who is British and is professor of geometry at Oxford University. This year's only prize for Islamic/Arabic studies went to Sheikh Abubakar Mahmud Gumi, Grand Mufti of Nigeria for translating the Koran into Hausa.

Atiyah's prize, given for his work in the area between geometry and theoretical physics, is the first to be awarded for mathematics, one of the four subject areas for which the science prize is given in rotation. Chemistry was the area last year, and Dr Michael Berridge the winner. And in 1984 for physics, the prize was shared by Drs Gerd Binnig and Heinrich Rohrer,



Saudi winners: Sir Michael Atiyah (left) and Dr Barrie Russell Jones.

who won Nobel prizes last year. But in 1983, its first year, and in 1985, the science prize was not awarded, again "because nobody good enough was nominated", says Prince Khaled al Faisal, director general of the King Faisal Foundation which created the prize.

The medicine prize, now in its sixth year, has always found a winner. It is awarded each year in a different area of medicine chosen to be of particular relevance to developing countries. Malaria, diarrhoeal disease and viral hepatitis have been among past topics. This year's subject, chosen by last year's jurors, was prevention of blindness. Jones is cited for his contributions to the prevention and treatment of trachoma and onchocerciasis.

In particular, his work established that chlamydial infections are responsible for trachoma. More recently, he has been pressing for the provision of simple programmes of teaching personal hygiene and health to rural communities in de-

veloping countries. That, he says, is a far more effective way to tackle blindness, 80 per cent of which could be simply prevented, than first to build a swanky specialist eye hospital, like the King Kalid Eye Hospital in Riyadh.

The annual process of finding prize-winners bears some resemblance to the Nobel process. With few exceptions, candidates have to be nominated by institutions: the Royal Society of London and the World Health Organization were among the nominees of this year's prizes. Relevant nominations are then refereed by anonymous experts in the subject area. The experts' reviews and the primary and other material are then sent to the relevant jury of "leading international scholars".

Foundation officials deny that sex, religion or race are factors in picking the winners, and jurors see no evidence of it either. On the other hand, of 14 science or medicine prize-winners so far, none has been female or Jewish. A sample of comparable size from other major prizes might also contain no women but would be unlikely not to include a Jew. Peter Newmark

Argentina to be sole user of nuclear dump

London

£18

ARGENTINA'S proposed nuclear waste dump in the Sierra del Media area will not be 'internationalized', at least until the end of the century, according to Cesar Arias, a spokesman for the radiological and security protection office of the National Atomic Energy Agency (CNEA). Speaking on radio last month, he said that the decision whether or not to allow other countries to use the site is not due to be taken until the year 2000, and will then depend not on the CNEA but on the government of the day. Commenting on protests against the dump (to be sited 60 km from Gastre, in Chubut province), Arias said that the granite of the region made it one of the safest places possible. He dismissed the opposition as "normal", as, he said, nuclear energy had created among the general public "an aura of mystery, passion and even sex (*sic*)".

Hints that the dump would be made available to imported waste have been rife in Argentine political circles. One likely applicant to use the dump (if and when it becomes available) would be Brazil, where no less than 17 dumping sites (including some ocean sites) have been proposed and have been met with widespread opposition.

Vera Rich

Space museum exhibit 'escapes'

Washington

THE Smithsonian Institution's Air and Space Museum, one of the most visited of Washington's public museums, has come to the rescue of Magellan, the Venus radar mapping mission scheduled for launch in 1989. Magellan was to have been launched by the shuttle carrying a Centaur upper stage. But last June, NASA (the National Aeronautics and Space Administration) cancelled the shuttle/Centaur Program, forcing Magellan's managers to switch to IUS, the Inertial Upper Stage.

Because IUS is less powerful than the Centaur, weight had to be trimmed. A lighter upper-stage adapter was built, saving 127 kg, but this meant carrying out new structural load analyses. Typically, that would have been done with the prototype satellite, but that was already being used in the satellite construction. With no way to perform the tests, the launch date was in jeopardy.

Enter the museum's Voyager spacecraft exhibit. The bus on the satellite on display



was close enough to the Magellan bus to be used in the tests. So, in January, workers removed the bus from the exhibit, sending it by truck to Martin Marietta's Denver plant where it will be modified to fit Magellan specifications.

By using the Voyager bus from the museum, NASA saved approximately \$1 million. But Magellan science manager Neil Nickle says that if the museum had not cooperated, no amount of money would have made it possible to construct another bus and still complete all tests by the scheduled April 1989 launch date.

Exhibits rescued from the Air and Space Museum have been useful for a real space mission before. Last November, the Air Force launched the Polar Beacon Experiment and Auroral Research satellite with parts from a Transit 5A satellite that had been hanging in the museum (see *Nature* 322, 297; 1986). Joseph Palca

Money to flow again at NIH, but how long will it last?

Washington

THE National Institutes of Health (NIH) last week made a decision that will be music to their grant-holders' ears: money withheld from new grants awarded since the beginning of the year will be given back. But the respite may be brief. The plan to carry \$334 million forward from the 1987 to the 1988 NIH budget, which initially prompted NIH's frugal action, is still on offer. Even if the money stays put, funds will be short in 1987 and could be extremely short in 1988.

The money to be handed back was part of the \$334 million shift — known as extended availability — that would have lowered the 1987 budget to \$5,850 million, even though Congress had already made its appropriation for 1987 at \$6,184 million. For 1988, the Reagan administration requested \$5,535 million, but with the money held over from 1987 the actual amount available for grants would be very close to the previous year's levels.

Congress must pass legislation in order to comply with the extended-availability scheme. So far that does not seem very likely. Senator Edward Kennedy has described the scheme as "robbing Peter to pay Paul". NIH director James Wyngaarden says the institutes have been withholding funds from all research grants

since 1 January in order to spread the cuts over the months before 30 September, the end of the fiscal year. Individual institutes were responsible for decisions on how budgets were to be pared, but generally reductions were made across the board.

Some regarded the NIH decision to hold back funds as illegal. Approximately two dozen organizations, including the Association of American Universities, were considering filing a lawsuit forcing NIH to release the funds. But legal action now seems unnecessary. On 24 February, NIH received instructions from the White House Office of Management and Budget (OMB) to release the money. The OMB made it clear that NIH should not "defer or otherwise restrict" currently available money until Congress gave its approval. But OMB spokesman Ed Dale says holding back funds did at first fit in with OMB's plans, although the cuts were not to be across the board.

It is still possible that Congress will agree to take the \$334 million out of the 1987 budget. This could result in budgetary upheaval if all the savings had to come from money to be awarded in the final quarter of the year. But if the money is not shifted, the net result will be a 10 per cent drop in the NIH budget for 1988.

Joseph Palca

Award for high-flyers at annual SDI black-tie banquet

Washington

BUILDING a strategic defence requires more than hardware, it needs dedicated people as well. That was the message from Lt-General James Abrahamson, director of the Strategic Defense Initiative (SDI) Office, as he helped last week to celebrate the second annual Strategic Defense Initiative Technical Achievements Awards Banquet sponsored by the American Defense Preparedness Association (ADPA).

An appreciative crowd packed the main ballroom at the Washington Hilton to see this year's winners receive their laurels. Seated among the military brass and defence contractors was last year's winner of the ADPA Strategic Defense Award, James Fletcher, administrator of the National Aeronautics and Space Administration. This year's award went to Edward Teller, associate director emeritus of Lawrence Livermore Laboratories, and Major-General Robert Rankine, director of space systems command, control and communications for the Air Force.

The evening was full of patriotic sentiment. The presentation of the colours was

followed by the national anthem, and an invocation suggesting biblical precedents for a strong strategic defence. Dinner was steak and potatoes (filet mignon and Berny potato sticks); decaffeinated coffee was available for the fainthearted.

The awards for technical achievement went to the Delta 180 project, a successful attempt to intercept an orbiting, thrusting target by a thrusting interceptor from a starting separation of 200 kilometres. Runner-up awards went to a test last summer of a low-altitude rocket interceptor, and to Martin Marietta's rapid re-targeting and precision pointing (R2P2) facility in Colorado.

Abrahamson was reportedly upset at the name "R2P2" because of its similarity to the robot character in the movie "Star Wars", but he managed a smile when the award was announced.

The laboratory award went to a team at the Air Force Geophysics Laboratory that developed a new long-wave infrared radar. Runners-up were an experiment to generate a multi-gigawatt burst of microwave radiation, and miniature valves to be

Brenner homes in on the human genome

Dr Sydney Brenner, alternatively molecular biology's favourite son and *enfant terrible*, is to stake a personal claim in the sequencing of the human genome by recruiting a team of "six to twelve" people to carry out a mapping project.

Until recently director of the Laboratory of Molecular Biology of the Medical Research Council (MRC), and now director of the MRC's Molecular Genetics Unit at Cambridge, Brenner plans partly to finance the project with the £300,000 of research money that formed a part of his recently awarded Louis Jeantet Foundation Prize. Further funds, between £200,000 and £250,000 a year, are being provided by MRC's Cell Board as well as by other undisclosed sources.

The research unit will be based at the Babraham (near Cambridge) site of the Agricultural and Food Research Council's Institute of Animal Physiology (now rechristened the Institute of Animal Physiology and Genetics Research).

Brenner's objective is far from explicit, but appears to involve both the characterization of cells from different tissue types by the genes which are active in them and the use of techniques for copying messenger-RNA molecules into DNA so as to identify whole classes of cognate genes, which can then be placed on the human genome by molecular hybridization.

Brenner, who is a member of the US National Academy of Sciences committee on human genome sequencing, is robustly of the view that a successful project need not be as expensive as some of the first estimates of cost, exceeding \$500 million (over 10 years). On the other hand, he says that novel developments in data handling and sorting may be necessary, which is why he is taking an interest in parallel-array processors.

John Maddox

used in kinetic-energy weapons.

Although the evening's emphasis was on scientific achievements spurred by SDI, Teller admitted that SDI work dealt with only "a few essentially new proposals". But Teller believes advances in technology for unmanned spacecraft necessitated by SDI will benefit the scientific community. He speculated on the day when SDI sensors could be trained on interesting astronomical events, such as the supernova in the Large Magellanic Cloud (see *Nature* 326, 11; 1987 and see page 121 in this week's issue).

This was not a night for criticism of SDI, unless it was for Congress's failure to pursue the programme with enough vigour. Representative Jack Kemp (Republican, New York) got a standing ovation for his keynote speech urging deployment of SDI by 1993.

Joseph Palca

India's research council to undergo belated modernization

New Delhi

A REVAMPING of the Indian Council of Scientific and Industrial Research (CSIR) by an injection of young blood has been recommended by a committee set up 10 months ago by Mr Rajiv Gandhi, the prime minister. The committee has indicted the CSIR for its failure to help modernize Indian industry and has provided a new package of measures for invigorating its management and forging better links with industry.

Since he became prime minister, Gandhi has been critical of the functioning of CSIR, of which he is president. He feels that CSIR scientists have been reinventing the wheel rather than concentrating on mission-oriented projects or on technology to prepare India for the twenty-first century. A year ago, he showed his displeasure by replacing Dr S. Varadarajan, then director general of the CSIR, with Dr A.P. Mitra, a physicist who was previously director of the National Physical Laboratory. He then set up a seven-member panel headed by Mr Abid Hussain, a member of the Planning Commission, to review the work of the CSIR and to recommend changes to make it more responsive to the needs of industry.

The review is the fourth since CSIR was created, and the most critical. According to the committee, CSIR has lacked focus and direction and "has been unable to develop technologies which would meet even the most agonizing needs of our economy and society, let alone facilitate modernization". The major failure, it said, was the council's inability to transform scientific results in the laboratory into technology for industrial production.

In April 1986, CSIR had more than 1,000 projects under way. By putting emphasis on known products and processes, the council had failed to make a major impact. Declaring the CSIR system "unwieldy if not grotesque" the committee said that, by functioning like a department of government rather than as a society, it "has tended to foster hierarchy or bureaucracy and stifle creativity". The committee was also appalled by the "middle-age bulge"; most CSIR scientists are well over 40 years of age and have stayed simply because there has been no mechanism for firing the more unproductive scientists.

Lack of team work has also been criticized by the committee, which found the CSIR to be a "loose federation of different laboratories rather than a cohesive system working for concrete goals and specified objectives". The committee observed that the council and the industrial sector have acquired distorted images of each other;

industry believes that CSIR is incapable of useful and timely research, while CSIR believes that "industries prefer the soft option of importing proven technology".

The committee's chief recommendations are aimed at bridging this gap. It has proposed that six of the nine members of the CSIR governing body should be from outside the CSIR. The chairman of the governing body should be an eminent professional scientist rather than, as at present, the CSIR director-general, and the governing body would relinquish its present role of administrative watchdog and focus more on formulating policy guidelines. The research councils of each

First, get rid of the dead wood...



laboratory would similarly be reconstituted with members representing industry and consultancy organizations.

Some of the recommendations relate to personnel. The director's tenure would be fixed at six years, after which he would return to research work. The strength of non-scientific staff would be reduced by half in three years and promotion would no longer be automatic. The committee has asked CSIR to get rid of unproductive scientists by encouraging voluntary retirement on generous terms. To bring in new blood, CSIR should hire good scientists on six-year contracts "at salaries 50 per cent more than the usual". A scheme of visiting scientists and another of distinguished fellows have been suggested to improve the climate for research. And there should be an institute for retraining and reorienting the entire CSIR fraternity "which has been allowed to be denuded without being recharged".

All CSIR laboratories have been asked to take up research projects identified by users and to work together with private industry. The linkage would go beyond the customer-contractor relationship "through exchange of personnel and equipment". The link with public-sector companies should be ensured by associating CSIR technologists with the acquisi-

Respite for French researchers

London

RECRUITMENT of young French researchers, frozen since last June, is to start again in all disciplines but biology, the new science minister, Jacques Valade, has announced. His decision to renew appointments for life to France's leading research council, the Centre National de la Recherche Scientifique (CNRS), is seen by applicants for research posts as a major victory in their long-standing struggle with the government.

The move not only affects those eligible for the jobs available in 1987. It also clarifies the position of the 285 young scientists deemed eligible last year, who were briefly promised jobs only to find their appointments invalidated by government decree (see *Nature* 324, 9, 1986). The 285, after extensive lobbying by their pressure group, the "Collectif des Admissibles", have had their one-year temporary contracts extended for life. In acting to restore the posts of the 1986 *admissibles*, Valade has managed to break the legal and political deadlock in the CNRS caused by the decision of his predecessor, Alain Devaquet, to overrule the 1986 appointments and to overhaul the appointment procedure.

Some problems still remain, however. Appointments in the life sciences remain blocked because of alleged 'irregularities' concerning the representation of the medical teachers' union on appointment committees. A decision is expected soon from the French high court. The *admissibles* have vowed to continue their campaign for permanent posts until the problems in biology are satisfactorily resolved, but, in the words of one CNRS spokesman, "at least everyone can breathe again".

Neil Crombie

tion by industry of imported technology "right from the beginning".

CSIR has also been asked to earn a third of its annual expenditure through consultancy to industry or government: the remainder would continue to come from public funds. In turn, industry has been asked to pay 0.75 per cent of the value of its output. This money could be used by industry to sponsor projects in CSIR laboratories.

The committee did take note of the achievements of the CSIR. Industrial production based on technology licensed by CSIR is now about £250 million. Some 50,000 tractors and a range of modern pesticides have been manufactured using CSIR expertise, and novel catalysts and petroleum-refining processes developed at CSIR have enabled India to emerge as a licenser in this high-technology field.

K.S.Jayaraman

The route to arms control without the hardware

Washington

As current talks in Geneva on strategic weapons in Europe emphasize, arms control negotiations tend to focus on force reduction. But a new report, published under the auspices of the American Academy of Arts and Sciences and the Cornell University Peace Studies Program, suggests more attention must be paid to the "nonweapons aspects of national security policy".

The report examines the viability of present command, control, communications and intelligence (C³I) systems. If both the United States and the Soviet Union expect deterrence to remain the basis for preventing nuclear war, then command systems must be sufficiently secure to make the ability to retaliate credible. But the report suggests that vulnerabilities of the current command system make it "likely to be severely degraded, if not collapse" following a major nuclear attack.

Several factors have conspired to make stability harder to achieve during a crisis. Paul Bracken, professor of public policy at Yale University and one of the report's authors, says the complexity of the strategic battleground requires intelligent computer systems to make it comprehensible. But as financial markets' response to computerized trading practices have shown, such systems can precipitate large responses, especially as human judgement is eased out of the loop. One recommenda-

tion of the report is to improve communications systems for the president and his top advisers. Other recommendations include additional systems to assure communications within the military hierarchy and field forces, especially submarines equipped with nuclear weapons.

Hillman Dickinson, former director of command, control and communications systems for the joint chiefs of staff and another author of the report, says modernization of US C³I should have the highest priority in the strategic budget.

Cornell physics professor Kurt Gottfried and Richard Garwin, an IBM fellow at the Thomas J. Watson Research Center, organized the report. Garwin suggests that issues relating to C³I have not generated much interest because "thinking doesn't cost money", so there is no profit motive driving such discussions. The initial report is aimed at generating public and congressional interest in the topic. The report's suggestion of a 1,500-mile interdiction zone for nuclear weapons around Washington and Moscow may capture the public imagination. Such a zone would give Washington a warning time of 15 minutes of a nuclear attack, much more than is now available.

Joseph Palca

The report, "Crisis Stability and Nuclear War", is to be published in expanded form later this year by Oxford University Press.

Conflicting signals from LMC supernova

London

A JAPANESE/US collaborative team has announced the detection of a burst of neutrinos from the recent supernova explosion, SN 1987a, in the Large Magellanic Cloud (LMC). But the timing of the burst conflicts with the detection reported earlier by the Italian/Soviet collaboration working at the Mont Blanc neutrino observatory.

The neutrinos were revealed as 'electron events' — Cerenkov radiation from high-energy electrons — in the Kamiokande II experiment, a nucleon-decay and solar neutrino observatory comprising 3,000 tonnes of water, located 1,000 metres underground in the Kamioka mine, western Japan.

At 7:35 Universal Time (UT) on 23 February, about 18 hours before the first optical sighting of the supernova, the Kamioka detector recorded a burst of 12 electron events in the space of 13 seconds. The electron energies ranged from 7.5 to 36 MeV, and the first two events pointed back to the LMC. The remaining ten events are consistent with an isotropic velocity distribution,

but this could indicate that these events represent the passage of antineutrinos — which scatter off protons — rather than neutrinos. Although detailed statistics are not yet available, a member of the collaborative team said that the neutrino signal was "exceedingly clean". In the experiment's previous 1½ years of operation, no unexplained electron events with energies above 20 MeV had been detected, yet the burst contained three such events.

The Mont Blanc observatory detected its burst of five events about 4½ hours before the Kamioka detection. If both detectors were recording neutrinos from SN 1987a, the bursts should have arrived simultaneously; the discrepancy thus calls into question the reality of one or both detections. Unfortunately, a third observatory which might have detected the supernova neutrinos, at the Homestake mine in South Dakota, did not record any events at all — a result which is nevertheless consistent with the Kamioka detection because of the much smaller size of the Homestake detector (see p.135).

Laura Garwin

Oftel moves to help Mercury

London

FOREIGN telecommunication monopolies needing a British link to their international networks will be required to allocate contracts to the two competing carriers in the United Kingdom in the same proportions as their overseas traffic.

This new ruling is that of Oftel (Office of Telecommunications), the watchdog of the British telecommunications industry which has become concerned that the new company Mercury, which is competing with the giant British Telecom, once a state-owned monopoly, is given a fair chance to compete.

Mercury, a wholly owned subsidiary of the international group Cable & Wireless, was given its final operating licence in 1984. In the same year, British Telecom was 'privatized', allowing it to compete more effectively overseas and to forge partnerships outside the United Kingdom. But Oftel worried that the previous contacts in overseas carriage enjoyed by British Telecom because of its earlier monopoly, could freeze out Mercury from the international scene.

The ruling on overseas monopolies is in the form of a 'Determination' issued by Professor Bryan Carsberg, director-general of Oftel, to ensure "that overseas monopolist operators cannot use the competition between British Telecom and Mercury to gain an unfair advantage".

The terms of the operating licence of both the British carriers require that they agree to some formula to monitor international traffic. In the absence of an agreed code, Oftel has imposed its own.

The new rules are a further attempt to liberalize the UK telecommunications market. Mercury, however, will be protected from further competition — by government decree — until the end of the decade.

Oftel is little concerned, at this stage, about agreements between British operators and those overseas carriers with indigenous competing telecommunications companies. Nor are they concerned if such overseas partnerships have the effect of reducing costs and increasing the efficiency of the services. While a substantial proportion of British Telecom's revenue comes from overseas traffic, it should remain unaffected if the corporation continues to generate future traffic in the same proportions as it has in the past.

Many European telephone companies still enjoy a monopoly and would be scrutinized under this new ruling. The United States has liberalized its market while Japan is in the process of deregulation. Their telephone companies are likely to be least affected.

Bill Johnstone

Creationism in Queensland

SIR—The report (*Nature* 321, 89; 1985) by Tony Thulborn on the evolution controversy as it affects secondary biology teaching in Queensland is fundamentally in error. The chief error is the statement that the teaching of creationism "is officially included in the school science curriculum". The truth is that creationism was deleted from the Queensland science syllabus in 1983. A teacher wishing to include creationism, or any other deviation from the syllabus, in the high-school biology curriculum must receive approval for the work programme from the Board of Secondary School Studies acting on recommendations by the District and State Review Panels. In other words, not only is creationism *not* included in the official science curriculum but its inclusion as a local variation must satisfy two screenings before receiving the board's approval.

Thulborn's statement that Queensland science teachers "are now obliged to present the conflicting arguments for creation and catastrophism" thus bears no relation to the prescribed curriculum. His statement is a highly tendentious interpretation of remarks by the Minister for Education, Mr Lin Powell, concerning "balance" in the teaching of evolution. Mr Powell has spoken for himself in the letters column of this journal (*Nature* 324, 204; 1986).

Building on this fundamental error, Thulborn describes a school-room crisis. He reports the formation of the Australian Association for the Protection of Evolution at a rally called to combat the furious onslaught of creationism. The alarming threat is heightened by reporting the decision taken by the association to assemble "survival kits" for teachers facing the creationist scourge. This crisis exists entirely in the imaginations of Thulborn and a handful of his associates. The survival kits have never been issued, no doubt for the good reason that they are irrelevant to the life of science instructors.

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THULBORN REPLIES—My report about creationism in Queensland was neither erroneous nor fanciful.

An instruction to include creationism in the science syllabus was first issued to Queensland school principals on 30 November 1981 by the then Minister for Education, Mr William Gunn. In November 1983, as Hiram Caton observes, the Board of Secondary School Studies released a draft syllabus (biology) which made no mention of creation. Neverthe-

less, the present Minister for Education, Mr Lin Powell, insists that teachers should still heed the 1981 instruction. In 1984 he tabled that instruction (*sic*) in the Queensland State Parliament, in response to questions about the teaching of creationism in state schools (*Hansard*, 10 April 1984). One member of parliament noted that the minister "failed to confirm or deny if biology teachers were being forced to teach creationism" (*Sydney Morning Herald*, 12 May 1984). In 1985, Powell said that "there would be no change in the secondary science syllabus, and the 1981 recommendations still stood" (*Brisbane Courier-Mail*, 30 April 1985). And in a published letter he has stated that the 1981 directive was "to ensure that students are exposed to both creationism and evolution" (*Brisbane Courier-Mail*, 13 May 1985). It is therefore legitimate to conclude that Powell, in his official capacity as Queensland's Minister for Education, has instructed teachers to include creationism in the school science syllabus.

Caton describes elaborate procedures to monitor the content of the science syllabus. Those safeguards certainly exist, though they are sometimes circumvented or ignored. In fact they have been most blatantly abused by the minister himself: Powell's instructions about "balanced" presentation have been addressed directly to school principals, thus side-stepping the approved procedures whereby the science syllabus is constructed and maintained by the Board of Secondary School Studies. In any case, it is obvious that existing safeguards do not exclude creationism from science classes.

In May 1984, an informed source in the Queensland Education Department revealed that only half of the science curriculum was prescribed by the department. "The remainder is determined by each school, which has to submit a school work program to the Board of Secondary School Studies. That board has been partial to proposals to teach creationism" (*Sydney Morning Herald* 12 May 1984).

In the same newspaper article, a senior science teacher commented that "creationists now have an open hand to teach it [creationism] with the official sanction of the Queensland Government and many are doing just that. The obvious next step is for creationism to become compulsory teaching in Queensland schools, and we are perilously close to that now." In addition, a lecturer at the Brisbane College of Advanced Education confirmed that several schools were known to teach creationism, identifying one of them by name. Last year, one science teacher recounted his experience when he refused to introduce creationism in his biology classes: those classes were promptly handed over

to a teacher trained in Home Economics (*The Skeptic*, November 1986, p.20). He also mentioned that one of his colleagues was being harassed because his teaching of ancient history conflicted with literal interpretation of the Bible. In short, a few teachers are fundamentalists who will teach creationism regardless of any official prohibitions; and other teachers have been encouraged, pressured or plainly instructed to bring creationism into their science classes.

On 10 July 1986, the executive committee of the Australian Academy of Science issued a statement condemning the teaching of creationism in school science courses. That statement makes particular reference to Queensland. Similar action has been taken, or is being considered, by other scientific, educational and religious bodies throughout Australia. Recently the Continuing Education Unit at Queensland University offered a short course specifically designed to help local schoolteachers to deal with evolution and creationism in their classes; more than 70 people enrolled.

The publication of a popular book titled *Creationism, An Australian Perspective* (edited by M. Bridgstock and K. Smith, Australian Skeptics, Melbourne) has been resoundingly successful: the first two editions were sold out in a matter of weeks, and the third has just been released. The book is proving particularly useful to the many teachers who are understandably perplexed by the whole issue. I am pleased to report that the Australian Association for the Protection of Evolution made useful contributions to these and several other ventures.

TONY THULBORN

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Research assistants

SIR—I note that you continue to publish letters on the "plight of British postdocs". I write now to suggest a comparatively simple step that would at least remove one irritation. I refer to the use of the word "assistant". While it is not unreasonable to advertise for "research assistants" at the grade of postgraduate, it seems both insulting and inaccurate to refer to "post-doctoral research assistants". Surely those advertising are seeking people who should contribute originality to the solution to a problem and are not mere assistants.

We suggest that postdoctorals should in future be referred to as research scientists, research biochemists or research fellows.

P.N. CAMPBELL

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UK citations

SIR—In view of the publicity given to the perceived decline of British basic science (see for example the leading article 'British science over the hill', *Nature* 323, 655; 1986) the recently published list of the most-cited 1984 life sciences articles from the Institute of Scientific Information in Philadelphia (*Current Contents, Life Sciences*, 29, No. 49, 3–17; 1986) is of interest.

Of the 102 most-cited papers, Dr Eugene Garfield has found that 61 of the institutions in which the research work was carried out are located in the United States and 13 are in the United Kingdom. Countries with which the United Kingdom has recently been unfavourably compared in fact produce relatively fewer scientific papers in this 'immediate high-impact' category; only seven of the institutions are in France, four in Japan and one in West Germany.

The comparatively strong presence of the United Kingdom in this latest record of trends in the life sciences seems not merely attributable to excellence in one or two areas, as the highly cited British papers covered very different topics such as growth factors and oncogenes, intracellular messengers, ion channels and secretion, transition metals in disease, antibodies in AIDS, renal transplantation, human complement genes and leishmaniasis. In view of the recent attacks on the British university system, it may also be worth pointing out that most of the highly cited UK papers came from research groups working in universities.

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On the defence

SIR—I have a certain sympathy for Alvin Weinberg's preference (*Nature* 324, 610; 1986) for a "defence-dominated" regime with vastly reduced nuclear arsenals. But I am not optimistic about its ultimate feasibility, as more than just scientific questions are involved. The United States and the Soviet Union would also have to trust each other enough to agree to release each other from the mutual hostage relationship of nuclear deterrence.

The Reagan administration's current unilateral and aggressive pursuit of strategic defences — the Strategic Defense Initiative (SDI) — seems almost designed to prevent this. The Soviets clearly view SDI as an attempt by the United States to achieve a first-strike capability and will redouble their efforts to maintain their deterrent force, they are hardly likely to respond by reducing their nuclear arsenals. A defence-dominated world cannot

be imposed unilaterally but can arise only through mutual agreement after a long process of disarmament and the development of some degree of trust among all the nuclear powers.

Weinberg may be right to complain that the Cornell survey of the National Academy of Sciences members' views on strategic defence failed to offer the possibility of a defence-dominated world. Taking these political considerations into account, however, I doubt whether many members of the academy would have given it high prospects.

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SIR—I read Alvin Weinberg's letter (*Nature* 324, 610; 1986) with care but find his argument for supporting the Strategic Defense Initiative (SDI) difficult to follow. He poses the question "...if arms control leads to a mutually agreed reduction in the offensive threat to, say, a few hundred offensive missiles, can a defensive system be effective against this much lower threat? The answer to this question is probably yes..." The essential word in this quotation is "if". Can one reasonably expect the Soviet Union to agree to reduction in missiles to a level that renders its offensive capacity negligible in the face of a deployed anti-missile system — in effect, unilaterally to disarm?

The Soviet Union is likely instead to seek parity, not in nuclear warheads or launchers, but in *deliverable* warheads. Thus any success in developing SDI by the United States will almost certainly be matched by an increase in nuclear missiles by the Soviet Union. It is true that this situation may be modified if the Soviet Union itself develops and deploys an anti-missile system on the scale of SDI but the inevitable temptation then for the United States will surely be to increase its own weapons to ensure that it maintains what it considers to be an adequate deterrence.

The history of warfare has been a constant competition between offensive weapons and defensive systems with developments in one serving as a stimulus for the other. Why should we suppose that the response to SDI should be any different?

D.W. MASON

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SIR—Alvin Weinberg's letter (*Nature* 324, 610; 1986), criticizing the Cornell Survey of the opinions of National Academy members of the Strategic Defense Initiative (SDI), has a basic flaw that invalidates his argument. His criticism is based on the expectation that SDI may be effective against an attack by a few hundred missiles rather than the several thousand now

possessed by each side and that missile reduction should therefore be sought. Weinberg proposes that critics of SDI should work towards such a reduction *and* for SDI as a means of stabilizing arms reduction.

The Reykjavik meeting brings out the inconsistencies of Weinberg's argument. The prospect of a substantial missile reduction collapsed over Soviet concern with SDI. That concern is probably based on Weinberg's thesis that SDI will be effective against the reduced missile threat rather than the present Soviet missile force so that a large reduction would leave the Soviet Union at a serious disadvantage.

A second thesis for Soviet concern would be an inability to respond to a US first strike. To many of us, such an action may not seem likely, but the situation could appear different to Soviet leaders. The repeated pronouncements of US presidents, from Truman on, on the use of nuclear weapons, and the repeated rejection by the United States of a 'no first strike' policy, would make the Soviet Union oppose an SDI programme that would prevent a Soviet response to a US attack.

Consequently, SDI and nuclear missile reduction are not incompatible, as Reykjavik demonstrated. Thus, Weinberg's criticism of the Cornell survey is not justified, and his questioning of the scientists motives is inappropriate.

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Shades of 1968

SIR—Under the headline "Shades of 1968?" (*Nature* 324, 500; 1986) one reads that "just why General de Gaulle's government took such fright...has never been crystal clear".

Some clarity may be provided by a slightly different statement of the facts.

The students did not protest against a "modest package of educational reforms". They were chanting slogans such as "*l'imagination au pouvoir*" a vague claim — although unreasonable — if ever there was one.

Then the whole country came to a stop when strikes paralysed industry, public services and the administration. Elections were called, a new government was formed and for the first time Edgar Faure took charge of the Ministry of Education. He then had a new law voted that led to what might appear as sweeping changes rather than "anodyne reform".

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Archaeological split

SIR—Much has been made of the 'great success' that attended the breakaway 'World Archaeological Congress' held at Southampton in September 1986. According to information one of us has received from the chairman of that meeting, "over 1,000 people registered from about 100 countries".

It is important to place these figures in perspective. The attendance of 1,000 contrasts sharply with the 2,000–3,000 that the organizers originally expected. Moreover, we would remind our colleagues that at the IXth Congress of the International Union of Prehistoric and Protohistoric Sciences (IUPPS), held at Nice from 13 to 18 September 1976, which, in accordance with the constitution of IUPPS, was open to scientists from all countries, 3,127 participants from 94 countries were present.

So, at the price of holding a meeting by sacrificing a major principle, the organizers gained five or six countries, lost 2,000 participants and did grievous harm to British and world archaeology. Add to that the unprecedented split in British and world archaeology, the wedge driven between colleague and colleague, the stimulus given to a major cleavage and politicization of world sciences ... and we cannot see that all this adds up to 'a great success'.

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Throwing stones?

SIR—Your correspondent Vera Rich never misses a chance to revel in *schadenfreude* at any misfortune that befalls the Soviet Union, which she immediately attributes to inadequate planning or ability to cope by the authorities. The latest outburst was her report (*Nature* 325, 188; 1987) of the spell of very cold weather that hit most of Europe between 11 and 16 January.

I have visited the Soviet Union three times during very cold winters and was impressed by the way buildings (including private homes) were heated, streets and railways kept free of snow. All forms of public transport ran efficiently, and diesel vehicles had preheated fuel and oil.

I was in England during this year's cold spell and was appalled by the inability of the authorities there to cope. Many roads, including some motorways, were closed, there was chaos on the railways and old people were dying of hypothermia in their homes, all this at temperatures 30° C above what the Russians were experiencing. The military managed to overturn a vehi-

cle carrying a nuclear weapon, an event that might well have developed into a catastrophe of Chernobyl proportions.

Maybe there is some Vera Rich in the Soviet Union churning out pejorative copy about the inability of the capitalist authorities to cope with a spell of only slightly cold weather, and their callous disregard of the plight of their citizens. But what such a correspondent would write could genuinely be classified as *pravda*.

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Space colonization

SIR—Von R. Eshleman's reservations about the near-term feasibility of space colonization deserve attention (*Nature* 324, 115; 1986). Colonization historically has been successful when existing technology permitted the migration of substantial numbers of people into large uncultivated areas that were suitable for agriculture. Conversely, experience has shown that the exploitation of a hostile environment for commercial or scientific purposes does not result in colonization. For example, the mapping of the ocean floor and offshore oil drilling have not resulted in any noticeable increase in the population of the oceans.

The rest of our Solar System is not suitable for agriculture. If the colonization of 'space' is judged to be a worthwhile goal, one way to proceed would be to use remote sensing devices to identify Earth-like planets in other Solar Systems. Once an Earth-like environment has been identified, it would be appropriate to initiate plans for colonization. An attempt to colonize the rest of our Solar System would probably be as cost effective and as successful as the European colonization of Greenland from AD 986 to 1599.

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More vaccine needed

SIR—The article by Tim Beardsley describing the formation and goals of the Task Force on Hepatitis B Immunization (*Nature* 324, 399; 1986) may inadvertently give the impression that we intend to achieve widespread hepatitis-B virus (HBV) immunization in high prevalence regions of the world solely through use of the vaccine produced by the Cheil Sugar Co. in Seoul, Korea.

That company deserves credit for having produced a pure and potent vaccine that can be manufactured at relatively low cost. Moreover, because of the generosity and humanitarian spirit of Mr B.C. Lee, chairman of Cheil's parent company, SAMSUNG, it is the first to commit itself

to providing vaccine for public sector mass immunization programmes at \$1.00 per dose, the maximum cost for a vaccine to be considered for inclusion in mass immunization programmes.

But the actual need for HBV vaccine throughout the world will be several hundred million doses a year, so it is clearly necessary for many producers to share responsibility for meeting these needs.

The Task Force is therefore in communication with all potential manufacturers of HBV vaccine, from whom we hope to be able to elicit similar commitments.

The Task Force favours widespread use of all safe, effective and affordable vaccines so that hepatocellular carcinoma and cirrhosis in high prevalence regions of the world can be controlled.

ALFRED M. PRINCE

(Chairman)

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Valuable libraries

SIR—As users of the human chromosome-specific libraries prepared by the Los Alamos and Livermore National Laboratories of the Department of Energy and distributed by the American Type Culture Collection, we wish to refute the suggestion that these libraries are not of value to molecular geneticists (*Nature* 323, 660; 1986). In our experience these libraries represent a valuable and freely available resource. Their "deficiencies" reflect a state of the art problem. There is no doubt in our minds about the "biological talent" of the scientists involved, nor of the usefulness of the libraries.

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Eiffel Tower threatens top astronomy

A French company's understandable pride in having built a conspicuous structure in Paris nearly a century ago may persuade it to ruin optical astronomy for several years to come.

Herstmonceux

WHEN the 4.2-m William Herschel Telescope comes into operation in the Canary Islands this summer, it will be the largest telescope to which British and Netherlands astronomers have access, and the third largest in the world. Far from city lights, it will be able to see the faintest galaxies. That, at least, was the original plan. But while people are putting the final touches to this fine instrument, the directors of the Eiffel Tower Company are contemplating a project that could seriously hinder its use, not to mention that of all other optical telescopes.

The intention of the *Société Nouvelle d'Exploitation de la Tour Eiffel* is to mark the centenary in 1989 of the Eiffel Tower with a display of space art. Having created a monument visible from all Paris, the company in its egotism wishes to create another visible from all the world. Unbelievably, this project is associated with the French Centre National d'Etudes Spatiales and the European Space Agency, even though the scientists in these organizations do not support it.

The Eiffel Tower Company has selected two proposals by competition. The front-runner is called the Light Ring, which would be launched to an altitude of 800 km in a 90-min orbit. At launch, the ring-structure of 2-cm tubes connecting 100 Mylar spheres each 6 m in diameter would be compacted into a cubic metre weighing 500 kg. After it was deployed, the heat of the Sun would evaporate 4 kg of water inside the structure, inflating it to a diameter of 24 km. It would appear as bright as 100 stars of magnitude +1 and would cover a patch of sky 0.5 degrees in diameter — the size of the Moon.

If this Light Ring passes over an optical telescope that is looking up, the observation will be ruined because light from the bright ring will totally swamp the natural starlight. If the telescope is feeding a sensitive amplified detector like the Image Photon Counting System (IPCS) in use on La Palma, the detector will be permanently destroyed, along with the phosphor and cathode of its sensitive image tube which is its main component. There are at present about ten suitable image tubes left for the IPCS in the world and each is literally priceless. It is not of course the only detector at risk. The area of sky swept out by the satellite in one pass over a telescope means that there is a one per cent chance that the telescope will accidentally view

the Light Ring on each pass — a virtual certainty that an accidental viewing would happen within a year unless precautions were taken.

The Light Ring would be visible from most countries of the world — that is its point. The whole of a summer's night and half a typical winter's night would be at risk.

The orbits of large, low-density structures are very uncertain because of the effects of atmospheric drag, and the pressure of solar radiation, including particle pressure which depends on solar activity. Errors of 15 min in an orbit predicted three weeks in advance are at present not unusual. The orbit of the Light Ring will be more uncertain because it is much larger than anything launched so far. Astronomers would have to invent ways to protect equipment from it all the time.

The proposers estimate that, with a 1989 launch date, but depending on sunspot activity, the Light Ring will last for three years before it is burnt up in the atmosphere.

If, on further study, the Light Ring poses insuperable technical difficulties, the Eiffel Tower Company proposes to select an alternative 'space art' project called Arsat. A curved reflecting sail would be launched into an 11-h orbit and would focus the Sun into a cross with a bright centre, projected on the Earth over a 3,000–5,000-km region.

From within the cross for three-quarters of an hour, Arsat would be visible as the brightest star, which would flash with a period of a few seconds as the sail slowly spins in its own plane, rotating the cross over a continent. Because Arsat would be focused, the satellite could be very bright. The project author calculates that it could perhaps be brighter than the Full Moon, flooding the sky with light. It would make it virtually impossible to carry out observations on faint stars while the satellite was above the horizon.

The costs of these two space art objects are in the range \$1–10 million, within the possible budget for a global celebration.

A more bizarre proposal than space art has come from the Celestis Corporation in Florida. It is to launch in reflective orbiting mausoleums the cremated remains of 15,000 individuals (compacted by a patent process to 3 cm³ each), in orbits that would last "for eternity". At \$50 million per launch, this is a profitable proposition.

The US Department of Transportation

(which is responsible for promoting the commercial space industry) has approved the Celestis Corporation's morbid project, which now depends only on market demand.

The effect of innumerable satellites visible to the naked eye orbiting over observatories would be to sweep bright trail after bright trail through a telescope's field. Already, virtually every deep photograph taken with the UK Schmidt Telescope in Australia over a 6 degree area of the sky is spoiled by an artificial satellite trail, those of the first and last two hours of the night having several trails. Even for the narrower half-degree field of a conventional telescope, the Celestis Corporation proposal has the potential to interfere with every image.

What of the future? Pierre Comte, the Arsat proposer, is an artist with *Leonardo*, a magazine devoted to the interface between art, science and technology. He writes: "Each time an event of worldwide significance takes place (Olympic games, international expositions, anniversaries important to our planet), it would be possible to announce it, to celebrate it by means of the presence of the Arsat satellites parading on the vault of heaven: a mobile star of extreme brightness, a group of stars forming an ephemeral and symbolic constellation. . . I simply tried to create an object that was beautiful, with pure lines and a dreamlike vocation: a star in the sky, but one born of human hands, a mad dream. . ."

Dreamlike or visionary this may, in a sense, be. But what a nightmare it is to anticipate a sky filled with orbiting trademarks or political symbols, offensive billboards perpetually overhead, from which there is no escape on Earth.

To counteract the space funerals, the International Astronomical Union in 1985 maintained that "no group has the right to change the Earth's environment in any significant way without full international study and agreement". These quiet and reasonable words do not give full force to the passionate feelings of astronomers on this issue. The noble, aesthetic aims of the space artists have been perverted in the egocentric proposals under consideration by the Eiffel Tower Company: the Celestis Corporation's materialistic proposals do not even have aesthetic virtue. The world's astronomers hope that these projects and others like them will be cancelled.

Paul Murdin

Organic geochemistry

Bigger and better hopanoids

Guy Ourisson

THE isolation of intact bacteriohopanetetrol, a precursor of most fossil hopanoids (microbial lipids), is reported by B. Mycke and colleagues on page 179 of this issue¹. The authors achieved this result by the rhodium-catalysed hydrogenolysis of kerogen (the insoluble organic fraction) from a 50-million-year-old shale. Their finding demonstrates that intact biological structures can be preserved in the high-molecular-weight organic components of sediments.

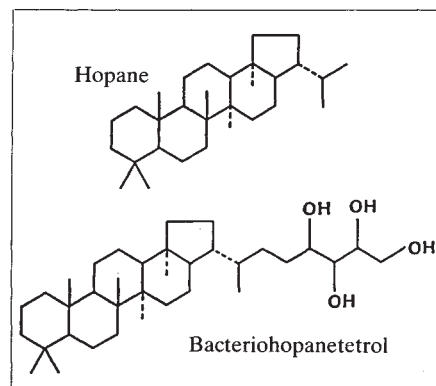
The Eocene shale studied by Mycke *et al.* is from Messel, near Darmstadt, a well-known palaeontological site. As well as very delicate fossils of snakes, fishes, crocodiles and other tropical vertebrates, Messel has also yielded an impressive list of molecular fossils, in particular of 'geohopanooids'. The discovery of bacteriohopanetetrol, derived from the C₃₀ triterpene skeleton of hopane by a five-carbon extension (see figure), increases by one the long list of these modified but recognizable derivatives of hopane found in sediments. Why is it remarkable that bacteriohopanetetrol has now been added to the list? To answer this question, I will first recount briefly the unusual story of hopanoids.

The first C₂₉ and C₃₀ hopane derivatives were discovered about 30 years ago in the resin of tropical trees Dipterocarpaceae,

and slightly more recently in ferns. In 1966, Hills and Whitehead described² the isolation of the first fossil hopanoid, a crystalline hydrocarbon obtained by careful fractionation of the high-boiling fraction of a Nigerian petroleum.

The advent of gas chromatography coupled with mass spectrometry allowed consistent, unambiguous syntheses, and over the next 10 years it became clear that geohopanooids are extremely varied, quite abundant and ubiquitous³. They have been found in all shales, clays, limestones, coals and petroleum products that are down to at least 500 million years old. They comprise stereoisomers (useful in evaluation of the maturation of sediments), aromatic or ethylenic hydrocarbons, alcohols, acids, methylketones, aldehydes and thiophenes. The C₃₀ pentacyclic skeleton of geohopanooids can be epimerized, rearranged, degraded to tetracyclic structures or cyclized further to hexacyclic structures. Most importantly, the skeleton can be extended by a straight chain of up to five carbon atoms, and maybe even a few more⁴. The abundance of geohopanooids is staggering: overall, their total mass is about 10¹¹⁻¹² tons, which is about as much or more than the total mass of organic carbon in all living organisms.

The accumulation and ubiquity of geohopanooids demanded more than an

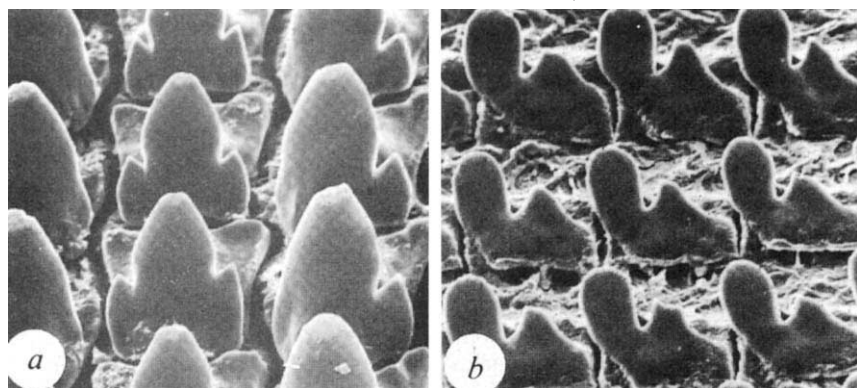


anecdotal explanation in terms of ferns or of odd tropical trees. An explanation was also required for the C₃₁–C₃₅ geohopanooids, for which no reasonable precursor was known: intuitively, one could accept that maturation in sediments could be accompanied by degradation or by rearrangements, but not by the stepwise addition of one to five carbon atoms as a straight chain.

The discovery⁵ of C₃₀ hopanoids in a few blue-green algae and bacteria suggested that the origin of these geohopanooids was microbiological, and this explanation became highly probable once bacteriohopanetetrol had been isolated from an *Acetobacter*⁶ and from a thermophilic bacterium⁷. This hopanoid was initially believed to be a 'special' metabolite, responsible in each case for a particular property of the microorganism producing it. But it rapidly became clear that it is only one of a large family of biohopanooids, and that these are extremely widespread, although not ubiquitous, among the most diverse bacteria⁸.

Since then, thanks mostly to the work of M. Rohmer's group⁹, the family of bacterial biohopanooids has been extended to include polyols, aminopolyols, aminocyclitol ethers, carbamoyl derivatives and even bacteriohopanetetrol-peptides and a C-nucleoside, 30-adenosyl-hopane. These complex hopanooids form a family of bacterial lipids of general significance, having in bacterial membranes the same structural role as cholesterol in eukaryotic membranes, and formed from squalene by the action of enzymes, the squalene-hopane cyclases, which are probably phylogenetic precursors of the epoxysqualene cyclases of plants, fungi or vertebrates^{10,11}. Bacterial hopanooids are "microbial lipids betrayed by their fossils"¹². The youngest, least-matured sediments contain the least-modified structures; it is therefore not surprising that bacteriohopanetetrol has been isolated from a recent soil and from bacterial mats. It is, however, rather reactive, which makes its isolation in a pure state difficult, and explains its many transformations in the inorganic matrix of the sediments.

The isolation of intact bacteriohopanetetrol in a 50-million-year-old shale by



AN almost ubiquitous molluscan feature is the radula, a toothed chitinous mouth structure used to pulverize food. The lower surface consists of many rows of minute teeth; the rasping oscillatory motion of the array of teeth against the floor of the mouth is sufficient to break up the chosen morsel, which can then be ingested. The electron micrographs show details of radular teeth of the land snail *Euryptyxis fragosa* (a, central and lateral teeth; b, marginal teeth, $\times 600$), and are taken from a paper by Peter B. Mordan in the *Bulletin of the British Museum of Natural History (Zoology)* 50, 207–271; 1986. This paper describes the southern Arabian Enidae, a group of non-marine molluscs, the detailed structure of the radula being an important diagnostic device in the taxonomic classification. The land snails are known to show a high degree of temporal distributional stability, and a comprehensive classification should eventually be useful in the biogeographical analysis of a region that, because it is located where three geographical zones coincide, has many population discontinuities. □

Mycke *et al.*¹ thus shows that complex, intrinsically reactive molecules can be efficiently protected by their inclusion (in this case as ethers) in the inert matrix of a heterogeneous polymer such as kerogen. It is remarkable that in the same Messel kerogen other highly informative fragments — the C₄₀ bis-phytanyl chains characteristic of the lipids of methanogenic archaeobacteria — had been isolated earlier.

It may seem surprising that the complex molecules isolated from sediments are often bacterial membrane constituents. But this certainly results from the fact that they are mostly derived from the terpene metabolism, and are therefore highly branched and biodegraded only with difficulty. In contrast, the major constituents of the microorganisms, proteins, reserve lipids and carbohydrates, are built to have a fast turnover: they are easily metabolized, either internally or, after death, by saprophytic microorganisms. It can now be assumed that the insoluble organic matter of other sediments (such as lignites

and coals) is largely derived from the most resistant constituents of the bacterial populations involved in the first stages of compaction, and that selective reactions will lead to the isolation of recognizable parts of structures. □

1. Mycke, B., Narjes, F. & Michaelis, W. *Nature* **326**, 179–181 (1987).
2. Hills, I.R. & Whitehead, E.V. *Nature* **209**, 977–978 (1966).
3. Van Dorsselaer, A. *et al. Tetrahedron Lett.* 1349–1352 (1974).
4. Ensminger, A. *et al. Tetrahedron Lett.* 3861–3864 (1972).
5. Bird, C.W. *et al. Tetrahedron Lett.* 3189–3192 (1971).
6. Forster, H.J. *et al. Biochem. J.* **135**, 133–143 (1973).
7. Langworthy, T.A. & Mayberry, W.R. *Biochim. biophys. Acta* **431**, 550–569 (1976).
8. Rohmer, M., Bouvier-Navé, P. & Ourisson, G. *J. gen. Microbiol.* **130**, 1137–1150 (1984).
9. Neunlist, S. & Rohmer, M. *Biochem. J.* **228**, 769–771 (1985).
10. Rohmer, M., Bouvier, P. & Ourisson, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 847–851 (1979).
11. Poralla, K. *FEMS Microbiol. Lett.* **13**, 131–135 (1982).
12. Ourisson, G., Rohmer, M. & Poralla, K. *Microbiol. Sci.* **4**, 52–57 (1987).

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Palaeontology

Life in the Cambrian seas

Desmond Collins

OUR knowledge and perception of Cambrian life has changed dramatically in recent years. One major contribution to this change was the re-study¹ of the Burgess shale fossils by H. B. Whittington which, besides confirming the predominance of arthropods, revealed many animals of unknown affinities. Now, another corner of the veil obscuring our view of Cambrian life has been lifted. On page 181 of this issue², S. Conway Morris and colleagues describe a soft-bodied Burgess shale-like fauna from the Lower Cambrian of Greenland. The fossils they describe are a

small collection of rather nondescript arthropods. Fortunately, as in Salome's dance, what lifting the veil suggests is more interesting than the view itself.

The Greenland arthropods lived 10 million years or more before the animals of the Burgess shale, and so demonstrate the evolutionary stability of the fauna. They also lived thousands of kilometres away from the Burgess shale basin (now in British Columbia), showing that the fauna was geographically widespread. The Greenland fossils described in this issue thus confirm the view of Whittington and

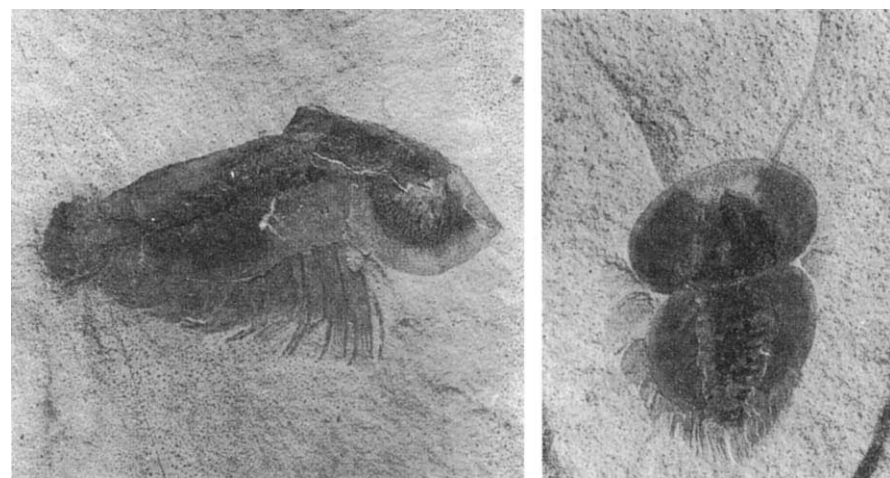
colleagues that the animals of the Burgess shale were typical of life in Middle Cambrian seas, and also demonstrate that such animals were probably typical of Lower Cambrian seas.

Specifically, the Greenland fossils repeat the preponderance of arthropods in the Burgess shale, particularly the dominance of soft-bodied arthropods over arthropods, such as most trilobites, with mineralized skeletons³. An additional fact, not emphasized in ref. 2, is the common occurrence of soft-bodied trilobites in this and other soft-bodied Cambrian faunas. The largest Greenland arthropod is similar to the soft-bodied trilobite *Tegopelte* from the Burgess shale, and another (Fig. 2e of ref. 2) looks very like *Naraoia* (see figure in this article), a soft-bodied trilobite found in the Burgess shale and in the underlying *Glossopleura* fauna⁴ of British Columbia, in the Middle Cambrian of Utah and the late Lower Cambrian of Idaho⁵, and in the early Lower Cambrian of China⁶. Soft-bodied trilobites, along with the other soft-bodied arthropods, seem to have been common animals in the sea for much of the Lower and Middle Cambrian epochs.

Sepkoski's statistical analysis⁷ of the origin and diversification of marine families in the Palaeozoic complements these changes in our perception of Cambrian life. Sepkoski observes that the pattern of diversification in the Lower and Middle Cambrian is appropriate for animals evolving to establish and fill ecological niches in the world's oceans. These animals had broad habitat and food requirements. Once competition for living space began in earnest in the Upper Cambrian, the generalized animals of the 'Cambrian fauna' declined and the more specialized animals of the succeeding 'Palaeozoic fauna' expanded. This interpretation is certainly consistent with the generalized organization of the Burgess shale arthropods, many of which do not fit any modern category, and with the great number of animals of unknown affinities in the Cambrian.

The study of Cambrian soft-bodied fossil faunas such as those in Greenland, British Columbia and elsewhere thus reveals the broadest range of 'body plans' that developed on Earth. Moreover, such fossils provide an exceptional insight into the evolution of life forms in conditions of minimal competition. □

1. Whittington, H. B. *The Burgess Shale* (Yale University Press, 1985).
2. Conway Morris, S., Peel, J. S., Higgins, A. K., Soper, N. J. & Davies, N. C. *Nature* **326**, 181–183 (1987).
3. Conway Morris, S. *Palaeontology* **29**, 423–467 (1986).
4. Collins, D. *et al. Science* **222**, 163–167 (1983).
5. Robison, R. A. *Paleont. Contr. Univ. Kans* **112**, 1–8 (1984).
6. Zhang Wen-tang & Hou Xian-guang *Acta palaeot. sin* **24**, 591–595 (1985).
7. Sepkoski, J. J. *Paleobiology* **5**, 222–251 (1979).



Naraoia, from Mount Stephen, British Columbia. Soft-bodied trilobites such as *Naraoia* and *Tegopelte* have now been found in Lower Cambrian faunas in Greenland, Idaho and China, and in Middle Cambrian faunas in British Columbia and Utah. (Compare with Fig. 2e of Conway Morris *et al.*² on page 182 of this issue.) The specimen on the left is 35 mm long; that on the right is 30 mm from tail to tip of head, excluding antennae.

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Evolutionary biology

Murderous mandibles and black holes in hymenopteran wasps

Paul H. Harvey and Linda Partridge

FOR the most part, biologists view adaptive evolutionary change as reversible. For example, when dark moths were selectively favoured through camouflage against a background of sooty tree trunks, it came as no surprise that lighter forms became more frequent again after the introduction of smoke-control measures. But there are instances of apparently irreversible evolution in which, following reversion to an ancestral environment, ancestral phenotypes do not re-evolve. For example, the transition from a mating system that entails high frequencies of inbreeding to one of outbreeding may be straightforward. But in an outbreeding population of diploid organisms, deleterious recessive genes can accumulate. The transition back to inbreeding now involves a selective disadvantage because the progeny of incestuous parents is more likely to receive a double dose of the deleterious recessive alleles. Charnov and Bull¹ have recently described such cases of irreversible evolution as involving 'absorbing states' which might be defined as evolutionary routes that, once taken, preclude return. (We would have preferred the expression 'black holes' so that an eminent astronomer could contribute for once to

evolution theory.) Recent work suggests² that a particularly gruesome absorbing state is involved in the distribution of gregariousness among species of parasitic wasps.

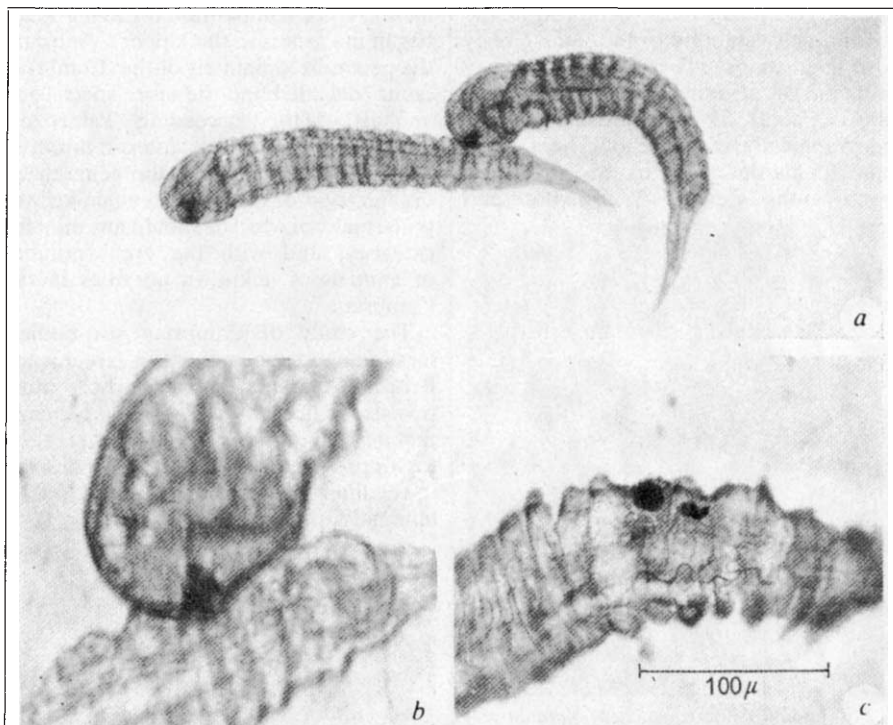
Apanteles, a braconid hymenopteran genus, contains more than 1,300 species which typically lay their eggs in lepidopteran caterpillars. The young feed on their host and emerge as it dies. Of 276 species for which data have been brought together by le Masurier³, more than half are solitary with a single young feeding on its host. Among the gregarious species, "there is a broad peak of brood sizes from 12–26, with some much larger broods (up to 1,200), but relatively few species with brood sizes in the range 2–11 larvae per host"³. Why are there so few species with 2–11 young per host? Among gregarious species, there is a strong correlation between host size and the total volume of the parasitoid brood. The absence of gregarious species with brood sizes 2–11 cannot be explained by a dearth of suitably sized hosts. These are available, but they are parasitized by solitary species. The answer seems to be more intriguing and involves mortal combat. Many of the solitary species possess long piercing mandibles

that are used to kill their fellow parasites in the same host. Salt⁴ describes the course of these fatal encounters — however many parasites are laid, just one survives.

Godfray² has now produced population-genetic models that throw light on the evolutionary dynamics of the process. Under what conditions would a gene for fighting invade a gregarious species? And when would a gene for cooperation spread in a population of fighters? If fighters are present in a brood, they kill all non-fighters and then attack each other until only one individual is left. Genes for fighting can spread whenever competition for resources within the host gets too intense, but mutant genes for tolerance in a population of fighters are soon done to death. In the simplest models, the actual condition for genes for tolerance invading a populations of fighters involves an Allee effect (individuals must have a higher fitness as one of a group than when alone). Once genes for fighting have spread, there is selection on mothers to reduce their clutch size to a single egg. The mandibles still provide a selective advantage because they can be used to kill the offspring of other mothers laying in the same host. And the production of solitary aggressive young can remain an evolutionary stable strategy even when the environment changes to favour larger optimal parental brood sizes. There is no point in a parent laying the larger optimal clutch size unless genes for tolerance have invaded the fighting populations and this, we have seen, is unlikely. Hence, argues Godfray, the strange bimodal distribution of clutch size in *Apanteles* is to be expected.

If Godfray is right, there should be solitary species that parasitize large hosts that might have been expected to provide nourishment for several parasites. Such species exist; when the parasites of large-bodied hosts are compared, the biomass of gregarious species per host is more than ten times larger than that for solitary species³. Also in agreement with Godfray's explanation is the finding that only solitary parasite species inhabit small-bodied hosts, whereas both solitary and gregarious species occupy larger hosts³. Once aggressive behaviour has evolved, which is likely to occur whenever parental optimal clutch sizes are reduced to two or three, it is very unlikely that tolerance, and hence gregariousness, will evolve again in that evolutionary line².

Analagous models for the evolution of siblicide in parasitic hymenoptera may also be applied to nestling birds. According to Godfray's work, the conditions for the spread of fighting and tolerant genes are the same for diploid and haplodiploid species. Siblicide is known in birds, but why is it not as common as in the parasitic hymenoptera? The occurrence of super-parasitism in the hymenoptera may be one reason — when more than one mother



Living first-instar larvae of the wasp *Nemeritis canescens*. a, b, Larvae fighting each other 72 hours after parasitization; c, melanized mandible marks, fifth day after parasitization. Magnification of b and c is about 3.5 times that of a. (From ref. 4, courtesy of Dr R. Fisher.)

typically lays her brood on the same host, the likelihood of a gene for tolerance spreading is reduced. Another contributing factor may be the typical avian hierarchy in size and fighting ability which facilitates the spread of tolerance².

Bull and Charnov¹ cite other examples of absorbing states, for example, once dioecy has evolved "ever-increasing specializations of males and females lead to the point at which both phenotypes cannot successfully function in the same individual". Herein lies one generality which the authors derive from their study of irreversible evolution. They point to the principle of accommodation. Once a state has evolved, the genome may progressively accommodate that state until reversible evolution is unlikely. The accumulation of deleterious recessive genes in outbreeding populations reduces the probability that inbreeding can be reinstated, and the production of aggressive larvae in small

clutches of parasitic hymenopteran larvae may effectively close the evolutionary route back to gregariousness.

The examples of irreversible evolution so far known involve some fundamentally different underlying processes. At present, it does not seem possible to predict the circumstances under which other cases might be found. However, it is now evident that evolutionary biologists must take their own version of black holes seriously — absorbing states in evolution exist, and they may crop up in the most unexpected places. □

1. Bull, J.J. & Charnov, E.L. *Evolution* **39**, 1149–1155 (1985).
2. Godfray, H.C.J. *Am. Nat.* (in the press).
3. le Masurier, A.D. *Ecol. Ent.* (in the press).
4. Salt, G.C. *Symp. Soc. exp. Biol.* **15**, 96–119 (1961).

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DNA structure

Tailors or machine operators?

Maxim Frank-Kamenetskii

PROGRESS is not necessarily contingent on the sophistication of equipment. People engaged in the DNA business have long been learning this lesson. Expensive ultracentrifuges have been ousted by trivial gel slabs as separators of DNA molecules. Now, apparently, it is the turn of nuclear magnetic resonance spectrometers and X-ray diffractometers to be rendered superfluous. These sophisticated machines are most efficient in studying the atomic structure of small DNA pieces under special conditions. But they are practically useless when one wishes to know what spatial structures the various parts of the natural DNA molecule adopt under conditions close to those inside the living cell. Among other things, the structure of a given DNA stretch may depend on ambient conditions including the surrounding sequence of nucleotides. This has now been convincingly demonstrated by K.M. Sullivan and D.M. Lilley (*Cell* **47**, 817–827; 1986).

Sullivan and Lilley used a very simple enzymatic method to detect cruciform extrusion in palindromic (invertedly repeated) regions in their DNA preparations. It has previously been shown that the two loops of a cruciform are preferentially attacked by the *S*₁ endonuclease, a process easily detected by gel electrophoresis. Sullivan and Lilley studied cruciform extrusion under superhelical stress in two different plasmids, one of which carries the ColE1 palindrome in its natural surroundings and the other carries the so-called bke palindrome inserted at the *Bam*HI restriction endonuclease site.

These two plasmids exhibit strikingly

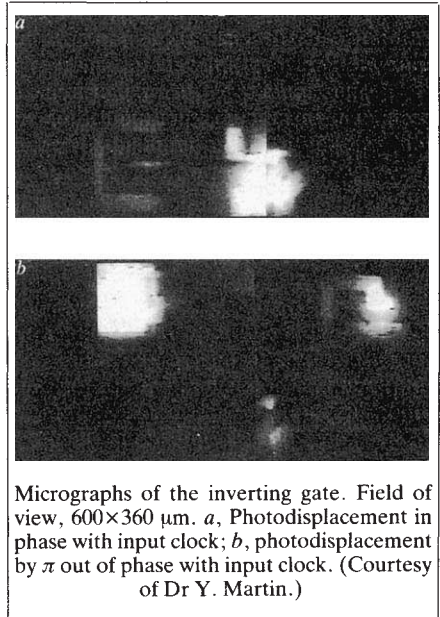
different behaviour with respect to cruciform extrusion as a function of ionic strength and temperature. This difference could arise from the differences in sequence between the two palindromes. Sullivan and Lilley tailored their plasmids so that the bke palindrome was flanked by ColE1 and the ColE1 palindrome was sewn into the *Bam*HI site. What did they observe? The answer was unambiguous: the behaviour depends on a nucleotide sequence that flanks the palindrome rather than on the palindrome itself. The anomalous behaviour of palindromes in the ColE1 surroundings probably results from the fact that the region is highly enriched in AT pairs.

The paper by Sullivan and Lilley is the most recent, but by no means the only, demonstration of the great advantages of the methods of genetic engineering in studying DNA structural polymorphism. Many people are altering natural DNA pieces, tailoring specially designed sequences, incorporating them into plasmids and probing the plasmids with enzymes and chemicals to investigate which alternative structures other than the canonical B-form various DNA sections can adopt under different conditions. The important lesson to be learnt from the work of Sullivan and Lilley is that the result of such probing may depend not only on the region itself but on the surrounding sequences as well. □

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Joule heat comes into its own

ON THE heels of the tunnelling electron microscope, and on the principle that it should be possible to construct a microscope of some kind around any microscopic phenomenon, two people at IBM's T.J. Watson Research Center at Yorktown Heights, New York, have now announced the development of the Joule displacement microscope, a device that can follow the flow of electric current in a microcircuit by the detection of the surface displacement brought about by Joule heating of the



Micrographs of the inverting gate. Field of view, 600×360 μm. *a*, Photodisplacement in phase with input clock; *b*, photodisplacement by π out of phase with input clock. (Courtesy of Dr Y. Martin.)

underlying semiconductors (Martin, Y. & Wickramasinghe, H.K. *Appl. Phys. Lett.* **50**, 167; 1987).

The essence of the technique is the use of a heterodyne laser interferometer, essentially a Michelson interferometer using two laser beams mismatched in frequency and which, by the use of the semiconductor surface as one of two reflectors, is less sensitive to drift of the optical path-length.

By way of demonstration, the system is used to follow changes in the topography of the surface of a semiconductor circuit (which required that the focused laser spot should be scanned across the surface), taken to be a commercially available inverter gate operating at 10 kilohertz. Because the heterodyne laser makes it possible to measure separately the in-phase and out-of-phase signals from the surface, it is possible to identify separately the pieces of the circuit which are expanded at different points in the cycle. The two photographs above show the images obtained when the phase angles differ by 90 degrees.

The obvious applications of the technique are to the study of the performance of developed microcircuits and the search for faults in prototype devices. □

Neurobiology

An eye on ancient circuits

Melody V.S. Siegler

EVERY nervous system has an evolutionary past and is very much answerable to it — such is the message of three recent papers that examine the rise and fall of neural circuits in flies¹, firebrats² and assorted other invertebrates³. In invertebrates, it is possible to recognize similarities between individually identifiable neurons, not only in different ganglia of the same species but also across widely separated families and orders. The authors of the three papers choose neurons for comparative study that are similar enough to be recognizable as homologues but different enough to be interesting. They conclude that these neural elements are essentially conserved but adapted and modified as new behaviours evolve.

Shaw and Meinertzhagen¹ ask how nervous systems might change during evolution to produce innovations in an animal's behaviour. They suggest two possibilities: either new types of neurons emerge that connect with and modify existing circuitry; or synaptic connectivity among existing neurons alters. They have studied the fine structure of the lamina, the outermost layer of the optic lobe, where photoreceptor axons synapse on precisely ordered arrays of identifiable postsynaptic neurons, in a range of dipterans, from ancient crane flies to modern houseflies and blowflies (see figure).

The lamina, along with the rest of the optic neuropile, has already been studied in exhaustive detail⁴ but never before, it seems, with an eye to at least 300 million years of evolutionary past. Shaw and Meinertzhagen conclude that all flies' eyes are not alike. They could recognize laminar neurons of more than a dozen uniquely identifiable types in each of the 14 species they examined. Moreover, the types seem to be homologous across the families, indicating a widespread conservation of neuronal form over time. What has changed is synaptic form, as revealed

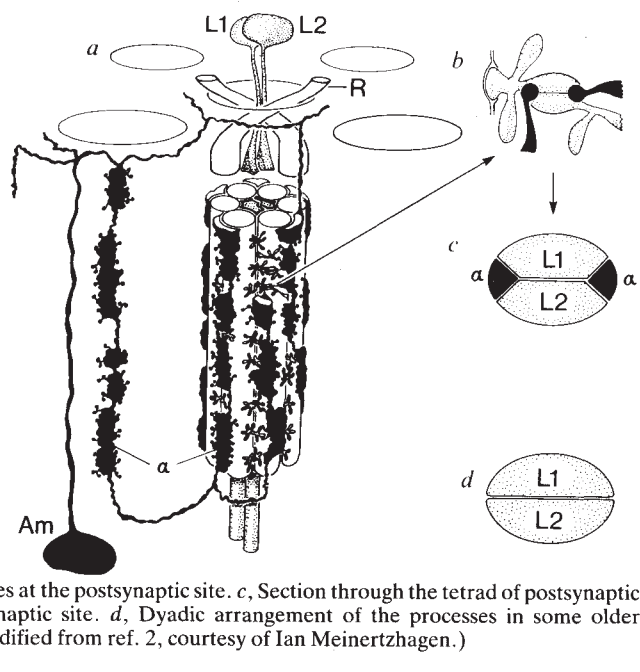
unequivocally in serial-section electron microscopy. Specializations of synaptic ultrastructure occur progressively in the phylogenetic sequence. These include elaboration of the presynaptic dense bars, imputed to be instrumental in the release of transmitter, and of the capitate projections of glial cells, possibly involved in membrane recycling.

Against this background of gradual synaptic change, there is one striking alteration in connectivity. Whereas in

the prototypes of an escape system elaborated in more advanced modern insects, cockroaches and crickets: a pair of specialized abdominal appendages, or cerci, that bear wind-sensitive sensilla, and several associated giant interneurons. The cell bodies, neuropilar branches and axons of the giant interneurons in the firebrat seem to be homologous to those of crickets and cockroaches — they are strikingly similar in shape and position and the terminals of mechanosensory afferents occupy comparable regions of the neuropile. And neuronal patterns in embryonic crickets and adult firebrats can almost be superimposed, an example of ontogeny recapitulating phylogeny.

Dumont and Robertson's review³ of some familiar neuronal circuits has a more

a, A lamina optic cartridge of the housefly, of the relatively recent family Muscidae, Diptera. Six photoreceptor axon terminals (R) converge around two large, monopolar neurons (L1 and L2) that run at the central axis of the cylindrical assembly. Small processes from L1 and L2 take part postsynaptically as a pair at many small, tetrad synapses on the surface of each presynaptic R terminal, together with two extensions that normally come from α processes (black) of lamina amacrine neurons (Am). *b*, Higher magnification of the postsynaptic processes at the postsynaptic site. *c*, Section through the tetrad of postsynaptic processes at the postsynaptic site. *d*, Dyadic arrangement of the processes in some older families of Diptera. (Modified from ref. 2, courtesy of Ian Meinertzhagen.)



lower flies each photoreceptor terminal synapses with two postsynaptic elements to form a 'dyad', in the higher flies dyads are replaced by the well-known dipteran 'tetrads', with an extra two postsynaptic profiles provided by pre-existing neurons (see figure). The tetradic arrangement, the authors conjecture, may be part of a local feedback loop that enhances the resolution of transient stimuli, important in the execution of the rapid turning movements in flight that are characteristic of houseflies and blowflies.

Among the most primitive of living insects are the wingless firebrats. Edwards and Reddy² have used these domesticated and cosmopolitan relatives of silverfish, denizens of bakeries and boilerhouses, to delve into the evolutionary past of insect behaviour. In particular, they ask how the wingless Devonian progenitors of modern insects evaded their terrestrial predators, spiders and scorpions. They find that firebrats have elements that seem to be

didactic aim — to free their fellow circuit-breakers from the ideal that nervous systems are optimally designed. Their analysis suggests that years of searching have yielded few satisfactory examples of simplifying 'organizational principles' linking circuitry to behaviour. Nervous systems do not live by parsimony alone, it seems: simple patterns of behaviour are controlled by complex circuits and similar problems are solved in many different ways. In the search for understanding, the past has been ignored too much. Some features of neuronal circuits may have no functional significance but may be merely vestiges of an earlier pattern of behaviour. Among the examples the authors give are the serially repeated interneurons in the abdominal neuromere of the locust metathoracic ganglia that influence a thoracic pattern generator; and the presence in all abdominal ganglia of crayfish of synaptic connections that seem appropriate only to some. These anachronisms persist

Erratum

In the picture story on aphids (*Nature* 325, 580; 1987) it is nepetalactone that gives catmint (*Nepeta cataria*) its characteristic smell, and not nepetalactol, as stated. The electron micrograph was taken by J. Hardie (Imperial College, Silwood Park).

Corrigendum

In last week's leading article on the object 1987a "Supernova almost on the doorstep" (*Nature* 326, 11; 1987) the words 'of this type' were inadvertently omitted from the sentence "Intriguingly, nobody has yet convincingly shown theoretically that supernovae of this type should occur at all".

because the animal has, so to speak, designed its way around them — old neurons have been incorporated into new patterns of behaviour or inappropriate connections have been overridden, though not necessarily replaced, by more appropriate ones.

Selection pressure acts on behaviour, not on neural circuits; as long as a circuit does its job, natural selection is powerless to pare it down. The difficulty, of course, comes in judging what has functional significance and what does not, given the limitations in our understanding of neuronal circuits. The evolutionary perspective

provided by Dumont and Robertson³ emphasises the value of comparative studies, such as those of Shaw and Meinertzhagen¹ and Edwards and Reddy², for providing the context in which these kinds of judgements can be made. □

1. Shaw, S.R. & Meinertzhagen, I.A. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7961–7965 (1986).
2. Edwards, J.S. & Reddy, G.R. *J. comp. Neurol.* **243**, 535–546 (1986).
3. Dumont, J.P.C. & Robertson, R.M. *Science* **233**, 849–853 (1986).
4. Shaw, S.R. *J. exp. Biol.* **112**, 225–251 (1984).

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Parasitology

Cloned schistosome antigens

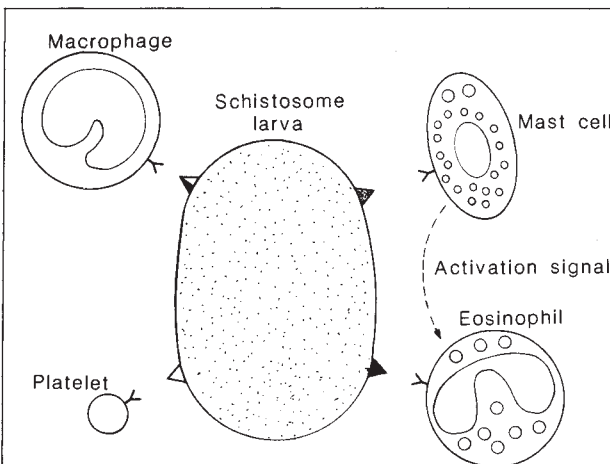
F.E.G. Cox

BECAUSE naturally acquired immunity to schistosomiasis is slow to develop and relatively ineffective, those hoping for a vaccine against this disease, which affects about 250 million people and countless cattle and buffalo, are looking at the possibilities provided by the techniques of gene cloning. After several false starts, this approach is now beginning to reap dividends. Professor André Capron and colleagues at Lille, France, in collaboration with a group at Transgène, report on page 149 of this issue¹ the isolation and expression of a gene, from adult *Schistosoma mansoni* worms, encoding an antigen that induces cytotoxic anti-parasite responses *in vitro* and protects a range of laboratory animals against schistosome challenge.

Schistosome infections begin when the larval worm bores through the skin and makes its way to blood vessels where it matures. While in the circulation, the worm acquires a variety of host antigens that effectively render it immunologically invisible, and there is therefore no protective response to the adult worm. But both larval and adult worms produce antigens that elicit immune responses to subsequently invading larvae, which are recognized and destroyed. This destruction is a complex process involving several kinds of immunoglobulin, including IgE, and several cell types, including eosinophils² (see figure). Vaccines must mimic this situation to be effective and it has been widely assumed that they would be based on larval antigens.

But the experiments described in this issue¹ take as their starting point an antigen of relative molecular mass 28,000 (28K) from adult worms³ quite distinct

from others with a similar molecular mass⁴. Capron and co-workers used RNA from adult worms to synthesize complementary DNA which, when injected into *Escherichia coli*, produces a 28K protein with the same electrophoretic mobility as



Immune responses in schistosomiasis are directed against the larval stage and involve macrophages, eosinophils, mast cells and platelets. Each cell type binds to a particular immunoglobulin which in turn recognizes a specific surface antigen. This results in the adherence of the cell to the schistosome larva and its subsequent destruction by antibody-dependent cell-mediated cytotoxicity.

the native protein. The recombinant protein contains 211 amino acids but for their experiments Capron and colleagues used a 172-amino-acid 25K fragment that comprises the immunologically essential component of the native protein. Rats and hamsters recognize this recombinant protein and respond by producing specific IgG and IgE antibodies which also recognize the native molecule. When tested *in vitro*, the IgE in conjunction with eosinophils is instrumental in killing schistosome larvae.

Capron and co-workers followed their success with *in vitro* studies with vaccination trials using the recombinant protein.

They found that it protected both rats and hamsters against challenge with schistosome larvae, shown by lower worm burdens in vaccinated animals compared with controls. Their final series of experiments demonstrates that vaccinated baboons produce cytotoxic antibodies similar to those found in rats and hamsters, suggesting that the vaccine is also effective in these primates.

The design of these experiments is important because rats and hamsters differ in their susceptibility to schistosome infections and the use of both species ensures that the results obtained pinpoint a fundamental mechanism of immunity. Baboons are the experimental animals most like man, so the successes achieved in all three species is encouraging when considering a human vaccine. Another encouraging feature is that the antigen used occurs in at least three other species of schistosome: *S. haematobium* and *S. japonicum* that infect man; and *S. bovis* that infects cattle, suggesting that the specificity of the vaccine is not too narrow. Successful vaccination of cattle against *S. bovis* using inactivated larval stages has already been achieved⁵ and it is to be hoped that the 25K protein will also be effective. It will certainly be easier to administer.

These results only point the way to a vaccine; much remains still to be done. Immunity to schistosomiasis is complex and at least four cell types have been implicated in larval killing (see figure). The experiments described here concentrate on the IgE–eosinophil mechanism and no role has yet been described for the IgG that is also elicited by the recombinant protein. But it is now possible to imagine a vaccine consisting of several antigens, each eliciting a specific immunoglobulin, which in turn might activate its appropriate cell type. Even if this kind of vaccine is not completely effective, it would probably reduce the worm burden in individuals and thus contribute to control measures. There are mathematical models⁶ that should

predict how efficient a vaccine against schistosomiasis would have to be for its use to be a worthwhile proposition. □

1. Balloul, J.M. *et al. Nature* **326**, 149–153 (1987).
2. Capron, M. & Capron, A. *Parasitol. Today* **2**, 69–75 (1986).
3. Balloul, J.M. *et al. Molec. biochem. Parasitol.* **17**, 105–114 (1985).
4. Taylor, D.W., Cordingley, J.S. & Butterworth, A.E. *Molec. biochem. Parasitol.* **10**, 305–318 (1984).
5. Taylor, M.G. & Bickle, Q.D. *Parasitol. Today* **2**, 132–134 (1986).
6. Anderson, R.M. & May, R.M. *Science* **215**, 1053–1060 (1982).

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Geophysics

A mechanism for reversals?

Mike Fuller

EVER since the demonstration of the reversal of the Earth's magnetic field, it has been hoped that this dramatic feature would provide a critical clue as to how the geodynamo operates. Another aspect of behaviour of the field studied with similar motivation is secular variation — the changes in the local geomagnetic field vector taking place over hundreds to thousands of years. Bloxham and Gubbins¹ have combined detailed studies of the recent field with a technique for projecting the surface observations downwards to produce a set of 'snapshots' of the field on the core-mantle boundary from 1715–1980. The approach is not quite as straightforward as the word snapshot suggests. Nevertheless, the work provides a new view of the geomagnetic field and has stimulated a reassessment of earlier ideas about secular variation. Now, on page 167 of this issue², Gubbins reports that the present decay of the dipole is caused by localized development of reversed flux in the Southern Hemisphere.

Gubbins relates this result to expulsion of toroidal flux by fluid upwelling, analogous to the processes in the Sun that give rise to sunspot formation and suggests that the growth and polewards migration of such reverse flux also provides a mechanism for reversals. His ideas are part of a new synthesis in geophysics, combining the efforts of seismologists and geomagnetists, which treats the Earth as a single system — a heat engine with events from the core to crust interrelated³. Thus, Gubbins suggests that the location of the fluid upwelling in the core is determined by the topography, or temperatures, at the base of the mantle, which is in turn controlled by mantle convection. These ideas have the virtue of being sufficiently specific to be testable against the palaeomagnetic record of reversals, as I will discuss.

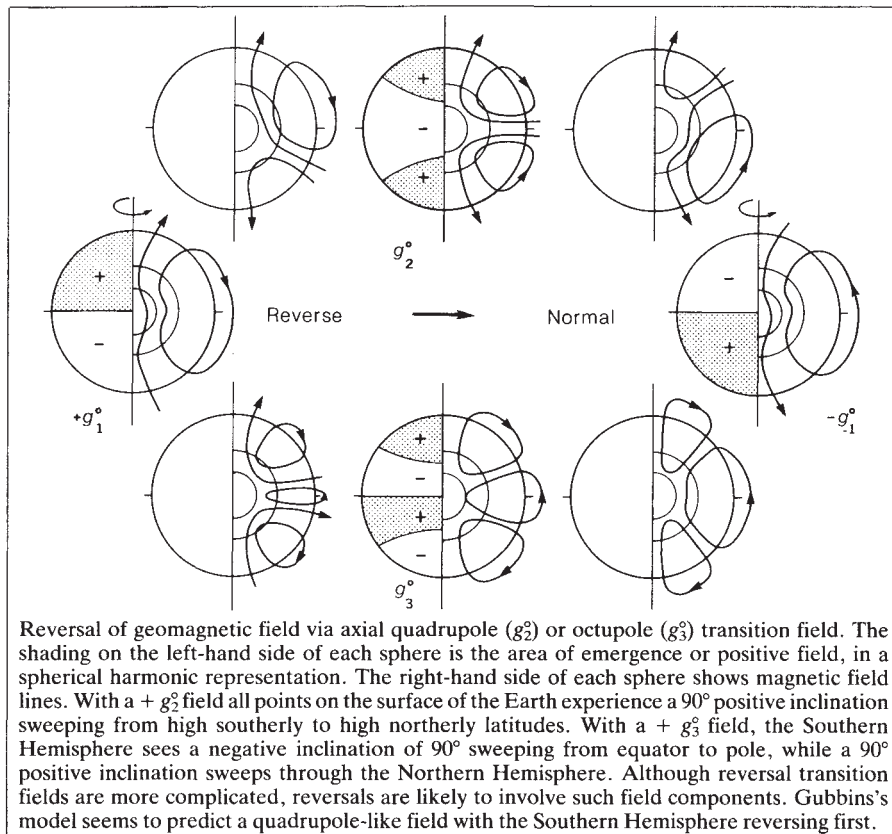
Once the dust had settled on arguments about whether the geomagnetic field had actually reversed, or whether the palaeomagnetic record of reversals was caused by aberrant behaviour of rocks, two broad avenues of research opened up. First, attempts were led by Allan Cox to determine the history of reversal rates. As McFadden and Merrill note⁴, there is now strong evidence for changes in the mean lengths of normal and reversed polarity intervals throughout geological time. Moreover, there is evidence that reversals are essentially a Poisson process. Thus reversals are a rare and random outcome of fluctuations in the state of the field in the core and are independent of each other.

The second area of research to emerge was the description of what happens when the field is reversing. Such palaeomagnetic records of reversals are hard to come by because the geomagnetic field spends a very short time reversing, compared with time in the normal and reversed states. Nevertheless, records have now been

and inclination during all reversals.

At one point, it appeared^{5,6} that the dominant spherical harmonic terms during the reversal might be low-order zonals. The dominance of these axisymmetrical harmonics explained how records of a reversal from sites at widely separated longitudes could all exhibit the observed high inclinations, as if the pole had passed beneath each site (see figure). Subsequent work points to the importance of non-zonal harmonics and to more complicated field morphologies.

There may yet be some worthwhile systematics to be gleaned from the records,



Reversal of geomagnetic field via axial quadrupole (g_2) or octupole (g_3) transition field. The shading on the left-hand side of each sphere is the area of emergence or positive field, in a spherical harmonic representation. The right-hand side of each sphere shows magnetic field lines. With a $+g_2$ field all points on the surface of the Earth experience a 90° positive inclination sweeping from high southerly to high northerly latitudes. With a $+g_3$ field, the Southern Hemisphere sees a negative inclination of 90° sweeping from equator to pole, while a 90° positive inclination sweeps through the Northern Hemisphere. Although reversal transition fields are more complicated, reversals are likely to involve such field components. Gubbins's model seems to predict a quadrupole-like field with the Southern Hemisphere reversing first.

obtained from lavas, intrusions and sedimentary rocks, and despite some discrepancies, a consistent picture of the major features is emerging.

The field reverses polarity in a few thousand years with an associated reduction in field intensity to about 10 per cent of the value before the onset of the reversal. This reduction in intensity may last for about 10,000 years: the field may recover its former intensity briefly during reversals, but this is a controversial suggestion.

There have been attempts to establish the global morphology of the field during reversals, but multiple records of the same reversal are available only for the last few reversals, so that the problem is daunting. The records from the last reversal show that the field was not dominantly dipolar, so that some repartitioning of the energy among the different harmonics clearly took place. This is also shown by the increased dispersion of values of declination

despite the failure of the simple zonal models. Even though we know that the field is not dipolar during the reversal, the systematics are most conveniently established in terms of virtual geomagnetic poles (VGPs) — the pole of the dipole field that accounts for the observed declination and inclination at a site. Indeed, the most direct evidence that the field is not dipolar during reversals is that the VGP paths for the same reversal from different sites do not coincide. Clearly they would if the field were dipolar. Using the VGP construction, it turns out that the paths are frequently confined within a narrow band of longitude. (If westward drift were an important part of the non-dipole variation during a reversal, this would not happen.) We have not yet been clever enough to interpret this observation. There are also now several examples from North America, western Europe and the Soviet Union of sites at which different

reversals have VGP paths confined to the same band of longitude. In one of the best-documented cases⁷, three reversals with related paths took place over a period of about 1.4 million years and then there was an abrupt change.

It is now possible to test Gubbins's model² in the face of the available data. The time taken for the reversal is so short that models relying on the decay of the dipole field must be discounted. The model passes the test; it invokes the generation of negative flux, which eventually overcomes the initial field. It is also consistent with a relatively long period of low field intensity before and after the field polarity actually switches. While the negative flux is generated, the dipole field intensity should decrease without major increases in worldwide secular variation. Indeed, this is just what is happening at the moment. But what is seen is not necessarily the onset of a reversal — on a statistical basis the best bet is that the dipole field intensity will again recover, as it has frequently in the past, without reversing.

Gubbins's model also fits nicely into the Poisson framework of reversal epoch lengths. As he notes, the dipole field will often have experienced reduction in intensity through development of reverse flux, but only on rare occasions will the field actually reverse. Changes in reversal rates are easily accommodated by changes in mantle convection patterns on the appropriate timescale of 10⁸ years.

The most critical test of the model comes from its prediction that all reversals should be similar within the lifetime of mantle convection patterns. Thus, the transition fields observed at particular sites should be similar. It is already known that reversals observed at the same locality are sometimes similar, but it is not clear whether they remain similar for the period required by the model. Preliminary indications are that the changes are too rapid to be consistent with it.

The constant location of the region of negative flux generation specified by Gubbins requires that reversals are all initiated in the same low-latitude region of the Southern Hemisphere. This is equivalent to a common starting point for a reversal model of the type suggested⁸ by Hoffman, based on the Busse dynamo⁹. Gubbins and Bloxham note¹⁰ that the core-mantle fields they calculate are similar to those predicted by Busse, although there are far more convection rolls in that model than predicted by the calculated fields. To the degree that Hoffman's model is successful in its predictions for the last reversal, Gubbins's model is also successful for that reversal, and it produces a mechanism to explain Hoffman's geometrical model.

Gubbins's model is not so obviously in conflict with available palaeomagnetic data on field reversals that it should be discounted. Unfortunately this, as is so

often the case in the Earth sciences, gives a sufficient, but not necessary, condition. Some might argue that the model must be rejected because it is not consistent with the frozen-flux assumption. Gubbins, on the other hand, holds that the data show the frozen-flux assumption to be incorrect on the reversal timescale.

Whether the model turns out to be successful or not, it has already stimulated renewed interest in the reversal process. It is rooted in observations made possible by new techniques of analysis of the present and recent geomagnetic field and challenges palaeomagnetists to come up with the necessary data for a critical test. Such a test requires new emphasis on obtaining

records of different reversals at individual sites, for which the best sources may well be ocean cores and rapidly deposited sedimentary sections. □

1. Bloxham, J. & Gubbins, D. *Nature* **317**, 777-781 (1985).
2. Gubbins, D. *Nature* **326**, 167-169 (1987).
3. Courtillot, V. *EOS* **67**, 809-812 (1986).
4. McFadden, P.L. & Merrill, R.T. *J. geophys. Res.* **80**, 3354-3362 (1984).
5. Hoffman, K.A. *Science* **196**, 1329-1333 (1977).
6. Williams, I. & Fuller, M. *J. geophys. Res.* **86**, 11657-11665 (1981).
7. Valet, J.-P. & Laj, C. *Nature* **311**, 532-555 (1984).
8. Hoffman, K.A. *Earth planet. Sci. Lett.* **44**, 7-17 (1979).
9. Busse, F. *Geophys. J. R. astr. Soc.* **42**, 437-459 (1975).
10. Gubbins, D. & Bloxham, J. *Nature* **325**, 509-511 (1987).

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Cell biology

The molecular basis for clathrin light-chain diversity

Dudy Bar-Zvi

COATED vesicles are involved in the transport of proteins between the organelles of eukaryotic cells and in receptor-mediated endocytosis, a specialized pathway by which cells take up specific molecules from the extracellular fluid. The coat of these vesicles has a characteristic polygonal structure of two protein complexes: assembly factor, composed of polypeptides of relative molecular mass 100,000–115,000 (100–115K) and 50K, and clathrin, composed of three 180K heavy chains and three 30–60K light chains, organized as a triskelion (refs 1,2; see cover picture). In two papers on pages 154 and 203 of this issue^{3,4}, Peter Parham and colleagues present data that help to elucidate clathrin light-chain structure and function.

There are several unexplained observations and unanswered questions about the function of clathrin light chains. Because the heavy chains alone can form triskelions that can self-assemble, it is not obvious that light chains are needed to generate a coated vesicle. They are required for the activity of the uncoating ATPase, a 70K heat-shock protein that catalyses the ATP-dependent removal of clathrin from coated vesicles *in vitro*⁵. Light chains can also stimulate the autophosphorylation of the 50K assembly factor polypeptide *in vitro*⁶. The significance of either of these activities *in vivo* is unknown.

With the possible exception of yeast, clathrin from other eukaryotic cells contains two distinct light chains⁷ (called α and β , a and b, or sometimes 1 and 2). Moreover, clathrin light chains from brain are about 3K heavier than the corresponding light chains from other tissues⁷. What do these differences in mass signify? Because light chains from brain and from other tissues compete for binding to the

same sites on the heavy chain near the centre of the triskelion^{8,9}; because either the α - or the β -light chains of brain can satisfy the requirements of the uncoating ATPase⁵; and because both bind calmodulin and calcium, the significance of the differences in mass is unclear. Indeed, all the light chains have common features, for example, they are all heat-resistant polypeptides and unusually sensitive to protease⁷.

Triskelions isolated from various tissues show variability in the molar ratio of the two light chains, ranging from 1 α -chain: 2 β -chains in brain to almost exclusively α -chain in reticulocytes. This variability, together with the observed difference in the mass of brain-specific light chains and those from other tissues, has prompted the suggestion that different specific light chains function in determining the specific properties or function of different types of coated vesicles. But because the polypeptide pattern and light-chain stoichiometry of endocytotic and exocytotic coated vesicles from liver seem to be identical¹⁰, there is no clear indication of why multiple but apparently functionally interchangeable light chains have evolved in most organisms. The only functional difference between the α - and β -light chains known so far is the differential phosphorylation of the β -chain by a coated vesicle-associated casein kinase II (ref. 11).

Knowledge of the molecular and structural details of clathrin light chains should help to elucidate their role and distinguish the functions of the different light-chain types. Jackson *et al.* in this issue³ report the cloning of clathrin α - and β -light chains from bovine brain and from lymphocytes. The α - and β -light chains are encoded by two closely related genes, so

that the amino-acid sequences of the two light chains are 60 per cent identical. Comparison of the nucleic-acid sequence for both kinds of clathrin light chains shows that the brain light chains contain a hydrophobic insert of 30 and 18 amino acids for the α - and β -chains, respectively. The new results also show that the tissue specificity of these polypeptides results from different splicing events.

Brodsky *et al.* in this issue⁴ map the different domains on the clathrin light chains using a panel of monoclonal antibodies. One region, residues 93–157, turns out to be inaccessible in native triskelions and coated vesicles, but accessible in free light chains. The authors therefore conclude that this region is involved in binding to the heavy chains. Another region, residues 158–208, is exposed in native triskelions and coated vesicles, and the authors suggest it is involved in coated-vesicle diversity. They also demonstrate that the brain-specific sequences are exposed in assembled clathrin, as expected.

Of great interest is the similarity in sequence of the carboxy-terminal clathrin light chains with the α -helical domains of various intermediate filament proteins³. Surprisingly, this homologous sequence, spanning about half the molecule, covers both the heavy-chain binding and the coated-vesicle diversity domains analysed by Brodsky *et al.*⁴, raising questions about the evolution of light-chain specificity.

These cloned genes should make it possible to explore the functional difference between the α - and β -light chains by shifting their ratio either by overproduction of one light chain or by introduction of anti-sense RNA resulting in the underproduction of one light chain. Furthermore, knowledge of which domains on the light chains are responsible for light-chain-heavy-chain binding and which domains can account for light-chain specificity should facilitate experiments using site-directed mutagenesis to elucidate further the role of clathrin light chains, the significance of two different light chains in each cell, and the role of the brain-specific inserts. □

1. Ungewickell, E. & Branton, D. *Nature* **289**, 420–422 (1981).
2. Pearce, B.M.F. & Bretscher, M.B. *A. Rev. Biochem.* **50**, 85–101 (1981).
3. Jackson, A.P., Seow, H.-F., Holmes, N., Drickamer, K. & Parham, P. *Nature* **326**, 154–159 (1987).
4. Brodsky, F.M. *et al.* *Nature* **326**, 203–205 (1987).
5. Schmid, S.L., Braell, W.A., Schlossman, D.M. & Rothman, J.E. *Nature* **311**, 228–231 (1984).
6. Pauloin, A. & Jolles, P. *Nature* **311**, 265–267 (1984).
7. Holmes, N., Biermann, S.J., Brodsky, F.M., Bharucha, D. & Parham, P. *EMBO J.* **3**, 1621–1627 (1984).
8. Winkler, F.K. & Stanley, K.K. *EMBO J.* **2**, 1393–1400 (1983).
9. Kirchhausen, T., Harrison, S.C., Parham, P. & Brodsky, F.M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2481–2485 (1983).
10. Helmy, S. *et al.* *Cell* **44**, 497–506 (1986).
11. Bar-Zvi, D. & Branton, D. *J. biol. Chem.* **261**, 9614–9621 (1986).

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Allan V. Cox (1926–1987)

ALLAN COX, Professor of Geophysics at Stanford University in California and principal architect of the magnetic polarity reversal timescale, one of the pillars of plate tectonics, died in an accident on 27 January. Shortly before his death he had announced his intention to return to teaching and research after nearly 8 years as Dean of Earth Sciences.

In the revolution of the Earth Sciences of the 1960s Cox, then at the US Geological Survey, played an essential role by determining and dating, in carefully chosen thick sequences of lava flows, the history of reversals of the Earth's magnetic field for the past several million years. With this work F. J. Vine and D. H. Matthews of the University of Cambridge were able to demonstrate that the oceanic crust of mid-ocean ridges faithfully records the magnetic reversals as it formed on ridge crests and was subsequently carried away laterally. Thus they confirmed the reality of the hypothesis of seafloor spreading, conceived a few years earlier by H. H. Hess at Princeton. Today the polarity-reversal timescale, extended now into the Mesozoic, has evolved to become a powerful chronostratigraphical tool of high resolving power and an essential part of the most recent geological timescales.

During the 1950s, work on the palaeomagnetic properties of ancient rocks had shown, largely through the efforts of S. K. Runcorn, then at Cambridge, that wegenerian continental drift was to be taken seriously as a possible explanation for the observed multiple polar paths. In the ensuing discussion older observations, indicating that the Earth's magnetic field had episodically reversed its polarity, attracted little attention. Cox recognized the potential importance of this process, providing that the reversals could be shown to be synchronous, world-wide and not caused by some process within the rocks themselves. Together with Richard R. Doell he succeeded in persuading the Geological Survey of the value of systematic further study of this phenomenon and began working on the project in 1959, soon joined by Brent Dalrymple who furnished the essential isotope dating.

In a very short time the three, together with the Australian team of Ian McDougall and D. H. Tarling, produced the first and already remarkably sophisticated magnetic-reversal timescale, just in time for Vine and Matthews to use it in sending the geological revolution on its way to victory in 1968. In brief essays accompanying a compendium of key research papers from this period (*Plate Tectonics and Geomagnetic Reversals*, 1973) Cox commented perceptively on the human as well as the scientific events.

In recent years, working largely with his students because university and national

science concerns made ever-increasing demands on his time, Cox turned to that other aspect of palaeomagnetism, the possibility of reading the original orientation and the latitude of formation of a rock from its magnetic inclination and declination. A life-long love for field work in the western United States did its part in determining his target, the geology of the collision zone between the North American and Pacific and associated minor plates. It turned out to be a superb choice because this work, now augmented by that of many others, has shown that our initial views of converging plate boundary processes were far too simple. The discovery that at convergence zones innumerable pieces of oceanic flotsam, 'exotic terranes' of often distant provenance, are aggregated and deformed, has returned to the mountain-building process that individuality of local character and history that plate tectonics in its first flush of pride had promised to erase.

Appointed in 1967 to the professoriate at Stanford, Cox was a teacher of great heart, capable of inspiring students well beyond the confines of geophysics. Quite often (and that may well be not as unique as our formal traditions pretend) he chose his subjects for research as much because of the people with whom he liked to work as by a coldly intellectual assessment of their potential.

Not surprisingly, he received many honours from his peers; among others the Vetlesen and Arthur Day medals, and memberships in the National Academy of Sciences, the American Academy of Arts and Sciences and the American Society of Philosophy.

As Dean of Stanford, treading carefully between its strong traditions of basic and applied Earth Sciences, he was instrumental in rejuvenating and invigorating the school. Balancing great respect for the judgement and advice of others with continuous insistence on high excellence and performance, he brought strength, youth and a forward look to its faculty and programmes.

Somewhat to the amazement of his friends, this shy and unassuming man also developed remarkable skill in fundraising. Deeply humane although somewhat reserved as he was, these administrative activities, not always fully understood by his colleagues, must at times have been a heavy burden to him, first undertaken from a sense of duty but later, one hopes, valued for the sense of accomplishment they provided.

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Neutrinos from the recent LMC supernova

SIR—The supernova explosion that was observed recently¹ in the nearby Large Magellanic Cloud (LMC) provides a unique opportunity to test the theory of neutron star formation in Type II supernova explosions. In particular, one can investigate the prediction that almost all of the gravitational binding energy of the residue neutron star ($\sim 3 \times 10^{53}$ erg) was radiated within a few seconds in the form of $\sim 10^{58}$ neutrinos with average energy ~ 10 –15 MeV. Here, we calculate the expected neutrino signals in terrestrial detectors which, according to the standard supernova theory^{2–10}, should have been observed during a short period (~ 1 s) before the onset of the optical supernova. (A measurement of the time delay between the first neutrino and optical pulses would be of great importance for our understanding of supernovae.) We also show how information on the fundamental properties of neutrinos (their life-times, masses and mixings, and their magnetic moments) can be obtained from neutrino observations of the LMC supernova. The number of neutrino-induced events in a terrestrial detector due to differential fluxes $d\phi/dE$ of neutrinos of types ν_i ($\nu_e = \nu_e, \bar{\nu}_e, \nu_\mu, \bar{\nu}_\mu, \nu_\tau, \bar{\nu}_\tau$) is given by

$$N = \sum_i n_i \int \frac{d\phi_i}{dE} \sigma(j, \nu_i, E) dE \quad (1)$$

where n_i is the number of particles of type j (atomic nuclei and electrons) in the target and $\sigma(j)$ are their cross-sections for relevant reactions which depend on the neutrino type and on the neutrino energy.

Recently Wilson⁷ and Wilson *et al.*¹⁰ have calculated the collapse of massive stars of 10, 15 and 25 $M_\odot$ and found that the predicted time-averaged neutrino signal does not vary greatly from star to star. In Table 1 we list the neutrino fluxes at the source as calculated by Wilson for a supernova explosion of a 25 $M_\odot$ star with a 1.6 $M_\odot$ iron core and the fluxes expected at a distance of 50 kpc from the supernova.

We have calculated the event rates in existing solar neutrino detectors using the spectra and fluxes of Wilson^{7,10} and neu-

trino cross-sections from Bahcall (ref. 11 and unpublished data). Kamiokande II¹², which contains 3 kt of water, should experience about 50 events with a positron recoil energy in excess of 10 MeV. The events should all be concentrated within a few seconds at an epoch that was a short time before the onset of the optical supernova on 24 February 1987. The high time resolution of the detector and the bunching in time of the real events should make the signal stand out above noise by a large factor. The 140-t liquid-scintillator detectors of the University of Pennsylvania¹³ in the Homestake mine and the 90-t scintillator detector in the Mont Blanc Tunnel¹⁴ should have seen about 3 events each if the standard supernova theory is correct. This signal is marginally detectable because of the high time resolution of the experiments. A comparison of the arrival times for neutrino events seen in different detectors will provide a crucial test of the physical reality of the observations; the neutrinos should arrive essentially simultaneously at all terrestrial detectors. For the ^{37}Cl tank of Davis¹⁵, only one additional ^{37}Ar atom is expected to be produced, a signal which would not be detectable.

Standard supernova theory may be wrong. It is more difficult to calculate how a star explodes than to estimate how the Sun shines and we do have the solar neutrino problem. To show the power of observations with existing neutrino detectors, we assume that 3×10^{53} ergs are emitted as thermal neutrinos (temperature T) and that the energy is distributed equally among all neutrino flavours. We find the following total number of events for the three different classes of neutrino detector mentioned above:

$$\text{events in 1 kt of H}_2\text{O} = 2.5 \left(\frac{T}{1 \text{ MeV}} \right) \quad (2a)$$

$$\text{events in 0.1-kt-liquid scintillator} = 0.35 \left(\frac{T}{1 \text{ MeV}} \right) \quad (2b)$$

$$\text{events in 0.6-kt } ^{37}\text{Cl detector} = 0.007 \left(\frac{T}{1 \text{ MeV}} \right)^{2.7} \quad (2c)$$

(The last relation overestimates the detection rate for temperatures above 5 MeV; it is too large by a factor of about 2 at $T=10$ MeV). A thermal spectrum with a typical energy of $T \sim 10$ –15 MeV would produce an event rate for the ^{37}Cl experiment that is an order of magnitude larger than the standard supernova model.

A number of authors, motivated by the solar neutrino problem¹⁶, have suggested recently that the neutrino has properties not included in the simplest electroweak model. Explanations of the solar neutrino problem of this type include neutrino decay¹⁷, resonant neutrino oscillations¹⁸ and a large neutrino magnetic moment (L.B. Okun *et al.*, preprint ITEP, 1986). The LMC supernova can be used to test

these suggestions.

If the ~ 10 MeV ^8B solar neutrinos decay significantly during their flight from the Sun to Earth, then essentially no neutrinos will survive the flight from the LMC supernova at a distance of 50 kpc. Consequently, the neutrino decay hypothesis which may explain the solar neutrino problem predicts no neutrino signals in underground neutrino detectors from the recent LMC supernova. If neutrinos are detected, their life times in the laboratory frame must exceed 10^5 years.

Neutrino oscillations increase the expected signals because the average energy of the ν_μ s and ν_s s is about twice that of the ν_e s (see Table 1). Electron neutrinos generate most of the signals and the interaction cross-sections increase with incident neutrino energy. If the ν_μ s and ν_s s oscillate into ν_e s (and vice versa) after leaving the neutron star (for example, through the MSW¹⁸ effect on positrons and electrons in the outer layers of the star) then the incident ν_e s will have higher energies and higher cross-sections and consequently will have more events in terrestrial detectors. Complete interchange between ν_μ s and ν_s s or ν_μ s will increase the signals in water and liquid scintillators by about a factor of two but will still produce only ~ 1 event in Davis's solar neutrino experiment.

Dar¹⁹ has shown that if the neutrino magnetic moment is in the range 10^{-10} to 10^{-12} Bohr magnetons (which explains the solar neutrino problem (L.B. Okun *et al.* preprint ITEP, 1986)), then relatively-high-energy neutrinos with a thermal spectrum can escape from a supernova explosion as a result of magnetic interactions with electrons. A conservative assumption, within this non-standard picture, is that the ν_e neutrinos are emitted with a temperature in excess of 10 MeV (and with about half the fluxes assumed for the thermal case cited above) (see ref. 19). The event rates for this model can be obtained from equation (2) by substituting the relevant temperature and dividing by two (to correct for smaller fluxes).

The collapse event produces a fluence of $\sim 3 \times 10^{58} (m_i/T)^{3/2} e^{-m_i/T}$ of neutrinos of flavours i with mass m_i . This flux contains a significant amount of energy if the neutrino mass, m_i , is less than a few hundred MeV. If these massive neutrinos are unstable and decay electromagnetically (see refs 20–22), for example, via $\nu_H \rightarrow \nu_e + e^+ + e^-$ or other electromagnetic decay modes, $\sim 10^{53} f (m_i/T)^{3/2} e^{-m_i/T}$ ergs will be produced in electron-positron pairs and γ -rays between the Earth and the supernova ($f=1$ if the neutrino life time, τ , is shorter than 5×10^{12} s, the flight time from the supernova to the Earth, and $f=\tau/5 \times 10^{12}$ otherwise). In order to observe an electromagnetic signal from this decay, it must take place outside the collapsing star

Table 1 Neutrino intensities and temperatures

ν Type	$N_\nu (\times 10^{57})$	$T(\text{MeV})$	$\phi_\nu (\times 10^{10})$
ν_e	4.5	4.7	1.6
$\bar{\nu}_e$	3.1	5.0	1.1
ν_μ	2.3	10.0	0.8
$\bar{\nu}_\mu$	2.3	10.0	0.8
ν_τ	2.3	10.0	0.8
$\bar{\nu}_\tau$	2.3	10.0	0.8

Number, N_ν , of neutrinos produced at the source and the flux (per cm^2), ϕ_ν , a distance of 50 kpc from a supernova in a 25 $M_\odot$ star with a 1.6 $M_\odot$ iron core¹⁰. Here T is the average temperature of the neutrinos in MeV.

($\tau \geq 10^3$ s). If more than $10^{-12} [f(m_i/T)^{3/2} e^{-m_i/T}]$ of this energy is converted to γ -rays, within one second (either by direct decay or by rapid electron-positron annihilation), we should detect a γ -ray burst coincident with the ν -signal. The absence of such a burst coincident with the ν -signal can be used to eliminate electromagnetic decay modes of neutrinos with masses in the range of one to a few hundred MeV and lifetimes larger than $\sim 10^3$ s.

Higher-energy neutrinos will arrive at the Earth before lower-energy neutrinos if the neutrino has a finite mass. However, this time delay, $\sim 0.01 (m_i/1 \text{ eV})^2 (10 \text{ MeV}/E_\nu)^2$ s, is too small to be observable for the LMC supernova.

A yet unknown, but potentially significant, fraction of the released energy might be emitted as gravitational radiation. The gravitational wave emission depends strongly on the asymmetry of the collapse and in particular on the amount of rotation of the core. Even with the most optimistic assumptions about gravitational radiation emission, $E_{\text{gr}} = 0.01 M_{\text{core}} \approx 0.016 M_{\odot}$, and $h = \Delta l/l \approx 0.7 \times 10^{-18}$, the LMC supernova is just below the sensitivity of current detectors. However, it is interesting to ask whether gravitational radiation would have been seen by next generation detectors (such as the proposed Caltech/MIT interferometer) with a sensitivity of 10^{-21} . At low angular momentum, the gravitational radiation emissivity scales²³ like J^4 . Therefore, the LMC supernova would have been detected even if the core was rotating only at 1/30 of its breakup speed, which is as slow as the Crab pulsar today. *Note added in proof:* Since this paper was

received on 2 March the neutrino burst was found by the Kamiokande experimental group, with properties generally consistent with the calculated expectations.

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1. Kunkel, W. & Madore, B. *Astr. Circ.* **4316** February 24 (1987).
2. Colgate, S.A. & White, R.H. *Astrophys. J.* **143**, 626 (1966).
3. Bethe, H.A. *et al. Nucl. Phys. A* **324**, 487 (1979).
4. Bludman, S. *et al. Astrophys. J.* **261**, 611 (1982).
5. Bowers, R.L. & Wilson, J.R. *Astrophys. J. Suppl. Ser.* **50**, 115 (1982).
6. Arnett, W.D. *Astrophys. J.* **263**, L55 (1983).
7. Wilson, J.R. *Numerical Astrophysics* (eds Centrella, J. *et al.*) 422 (Jones & Bartlett, Boston, 1986).
8. Bethe, H.A. & Wilson, J.R. *Astrophys. J.* **295**, 14 (1985).
9. Burrows, A. & Lattimer, J.M. *Astrophys. J.* **299**, L19 (1985).
10. Wilson, J.R. *et al. Ann. N.Y. Acad. Sci.* **470**, 267 (1986).
11. Bahcall, J.N. *Rev. mod. Phys.* **50**, 881 (1978).
12. Beier, E.W. in *Proc. 7th Workshop on Grand Unification, ICOBAN April 1986*, Toyama, Japan.
13. Cherry, M., Fowler, W.A. & Lande, K. (eds) *AIP Conf. Proc. No. 126* 32 (American Institute of Physics, New York, 1985).
14. Badino, G. *et al. Nouv. Cim. C7*, 273 (1984).
15. Davis, R. Jr. *Proc. 7th Workshop on Grand Unification, ICOBAN April 1986*, Toyama, Japan.
16. Bahcall, J.N. & Davis, R. Jr *Science* **191**, 264 (1976).
17. Bahcall, J.N., Petcov, S.T., Tashav, S. & Valle, J.W.F. *Phys. Lett.* (1986).
18. Mikheyev, S.P. & Smirnov, A. Yu *Nouv. Cim. C9*, 229 (1986).
19. Dar, A. *Phys. Rev. Lett.* (submitted).
20. Cowsik, R. *Phys. Rev. Lett.* **39**, 784 (1977).
21. Falk, S. & Schramm, D.N. *Phys. Lett. B* **79**, 511 (1978).
22. Dar, A., Goodman, J. & Nussinov, S. *Astrophys. J.* (in the press).
23. Shapiro, S.L. & Teukolsky, S.A. *Black Holes, White Dwarfs and Neutron Stars* (Wiley, New York, 1983).

Sulphated polysaccharide structures

SIR—Anyone with a knowledge of the actual structures of the molecules will have been amused by the debate between Lubec and Cole *et al.* on the validity of the use of heparin as a functional analogue of heparan sulphate in N-CAM-mediated cell adhesion studies, and the use of chondroitin sulphate as a control molecule in polysaccharide binding studies¹.

In their response, Cole *et al.* circumvent much of the structural argument but they do point out that, contrary to Lubec's claim, heparin does contain O-sulphate groups and so chondroitin sulphate is not such a bad control. Their case might have carried greater conviction had they not just mentioned the 3-O-sulphates but also pointed out that heparin actually contains a higher concentration of O-sulphates than chondroitin sulphate though these are present mostly as 6-O-sulphates and 2-O-sulphates. The 3-O-sulphate groups occur only rarely and in a minority of the heparin chains.

As to heparin and heparan sulphate, both are negatively-charged and com-

posed of an alternating arrangement of α , β -linked glucosamines (GlcN) and hexuronate. The GlcN units may be N-sulphated or N-acetylated. In heparin over 80 per cent of the GlcN units are N-sulphated and in total there are over two sulphate groups per disaccharide. In heparan sulphate there are approximately equal numbers of N-sulphated and N-acetylated glucosamine residues arranged in a predominantly segregated fashion. The O-sulphates are always in very close proximity to the N-sulphates, forming sulphate-rich domains which co-exist with non-sulphated acetyl-rich regions in the intact polymer. The sulphate-rich areas are heparin-like, though they rarely possess the sulphate density found in heparin^{2,3}. The key point is that the sulphate clusters are spaced-out along the polysaccharide chain. Heparin cannot reproduce this lateral asymmetry in polymer sulphation which is probably very important for the functional specificity of heparan sulphate. Nevertheless, because the sulphate groups in heparin are of a similar type to those in heparan sulphate, it is the best available functional analogue. It must also be stressed that heparan sulphate is

not a single entity. The content of O-sulphate groups is variable and shows some correlation with cell lineage and cell proliferation⁴. These variations are probably essential for the cell-type specific functions of the polymer.

Finally, Lubec is wrong to imply that heparan sulphates ensnare enormous numbers of proteins in an ionic embrace. They bind selectively to different types of collagens, they do not all bind to fibronectin and only a small proportion bind to anti-thrombin III. They are much too fastidious in their interactions to be referred to as molecules with 'glue-activity'.

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1. *Nature* **323**, 743-744 (1986).
2. Gallagher, J.T., Lyon, M. & Steward, W.P. *Biochem. J.* **236**, 313-325 (1986).
3. Lindahl, U. & Hook, M. A. *Rev. Biochem.* **47**, 385-417 (1978).
4. Gallagher, J.T. & Walker, A. *Biochem. J.* **230**, 665-674 (1985).

Alzheimer's disease and aluminium

SIR—At a time when an article in *Nature* can send securities plunging in exchanges all over the world (in this case the aluminium manufacturing companies' stocks), the opening paragraph of K. Tennakone and S. Wickramanyake's letter¹ is not nearly as cautious as it should have been.

The aluminium model for Alzheimer neurofibrillary tangles died² in the same journal issue it was born³ and kept a semblance of after-life for grantmanship reasons only. High levels of aluminium in senile plaques are not indicative of a causative role of the metal: the neurotoxic effects of aluminium bear no pathological resemblance to Alzheimer's disease, as has been shown in numerous studies of dialysis encephalopathy. And, studies of large kindreds with Alzheimer's disease⁴ show no environmental influence whatsoever on the incidence of the disease.

Persistent misunderstanding about the putative role of aluminium in Alzheimer's disease prompted me recently to publish a report of an old case of accidental metallic aluminium implantation in the human brain⁵: the ensuing clinico-pathological picture of intractable epilepsy with diffuse gliosis, without any lesion reminiscent of Alzheimer's disease, ought to put the matter to rest.

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1. Tennakone, K. & Wickramanyake, S. *Nature* **325**, 202 (1987).
2. Terry, R.D. & Pena, C. *J. Neuropath. exp. Neurol.* **24**, 200-210 (1965).
3. Klatzo, I. *et al. J. Neuropath. exp. Neurol.* **24**, 187-199 (1965).
4. Foncin, J.-F. in *New Concepts in Alzheimer's Disease* (eds Briley, M. *et al.*) 242-256 (Macmillan, London, 1986).
5. Foncin, J.-F. & El Hachimi, K.H. in *Senile Dementias: Early Detection* (eds Bès, J. *et al.*) 191-201 (Eurotext, London, 1986).

Transmission dynamics of HIV infection

Robert M. May and Roy M. Anderson

Simple mathematical models of the transmission dynamics of human immunodeficiency virus help to clarify some of the essential relations between epidemiological factors, such as distributed incubation periods and heterogeneity in sexual activity, and the overall pattern of the AIDS epidemic. They also help to identify what kinds of epidemiological data are needed to make predictions of future trends.

DESPITE remarkable advances in understanding the basic biology of human immunodeficiency virus (HIV — the aetiological agent of AIDS, acquired immune deficiency syndrome)¹⁻⁵ public health planning continues to be hampered by uncertainties about epidemiological parameters⁴⁻⁶. Accurate information about the typical duration and intensity of infectiousness, or about the fraction of those infected who will go on to develop AIDS (and after how long), will emerge only from carefully designed studies on these same timescales, which is to say many years. In the absence of such information, mathematical models of the transmission dynamics of HIV cannot be used at present to make accurate predictions of future trends in the incidence of AIDS, but they can facilitate the indirect assessment of certain epidemiological parameters, clarify what data is required to predict future trends, make predictions under various specified assumptions about the course of infection in individuals and patterns of sexual activity within defined populations (or changes therein) and, more generally, provide a template to guide the interpretation of observed trends^{7,8}.

Whether an infection can establish itself and spread within a population is determined by the key parameter R_0 , the basic reproductive rate of the infection⁷. R_0 is the average number of secondary infections produced by one infected individual in the early stages of an epidemic (when essentially all contacts are susceptible); clearly the infection can maintain itself within the population only if R_0 exceeds unity^{9,10}. For a sexually transmitted disease (STD), R_0 depends on c , which is essentially the average rate at which new sexual partners are acquired, on β , the average probability that infection is transmitted from an infected individual to a susceptible partner (per partner contact) and on D , the average duration of infectiousness^{7,11}. In what follows, we mainly restrict attention to the spread of HIV among

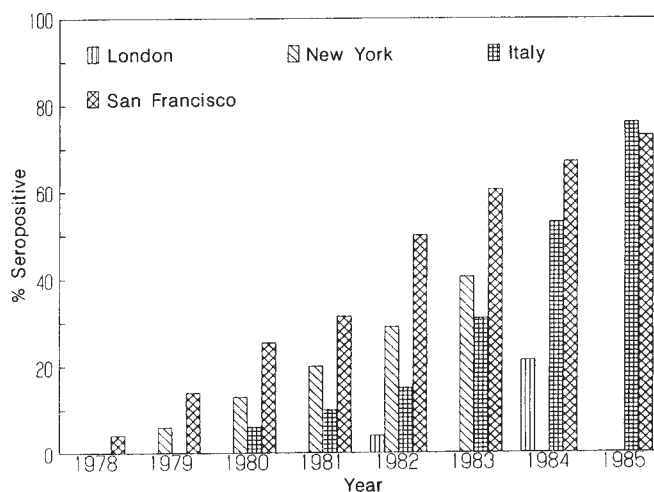


Fig. 1 The rise in seropositivity to HIV antigens in cohorts of patients over the period 1978–1985. The studies in San Francisco¹², London²³ and New York³⁵ were of homosexual/bisexual males. The study in Italy²⁴ is of drug addicts.

homosexual males, now responsible for the bulk of AIDS cases (about 70–80% in the United States, and a similar proportion in European countries^{6,12,13}).

Initial stages of the epidemic

The characteristics of most STDs cause their epidemiology to differ from that of common childhood viral infections^{11,14,15}. Unlike infections caused by airborne transmission, the rate at which new infections are produced is not dependent on the density of the host. Second, the carrier phenomenon in which certain individuals harbour asymptomatic infection is often important (as in the spread of herpesvirus). Third, many STDs induce little or no acquired immunity on recovery (for example, gonorrhoea) and fourth, net transmission depends on the degree of heterogeneity in sexual activity prevailing in the population.

As Hethcote and Yorke¹¹ have shown in their studies of gonorrhoea, mathematical models for the dynamics of STDs need to take account of the substantial variations of sexual activity within the population at risk. A particular risk group (such as male homosexuals in San Francisco¹²) of total size N may be roughly partitioned into subgroups of size N_i , each of whom on the average acquire i new sexual partners per unit time (when $N = \sum_i N_i$). The probability

that susceptible individuals in this i th group will become infected, per unit time, is thus $i\lambda$, where λ is the probability that infection is acquired from any one new partner. In turn, λ is equal to the product of the transmission probability β defined above and the probability that any one randomly-chosen partner is infected (with such partners being more likely to come from the sub-groups of individuals with high degrees of sexual activity).

Exponential growth

When these assumptions are incorporated into a model for the transmission dynamics of HIV infection, the infected fraction of the population at risk (who are seropositive in tests

for HIV) rises exponentially, as $\exp(\Lambda t)$, in the early stages of the epidemic. The exponential growth rate, Λ , is related to the basic epidemiological quantities defined above by:

$$\Lambda = \beta c - 1/D \quad (1)$$

The effective average over the distribution by degrees of sexual activity, c , is given explicitly as

$$c = \Sigma_i^2 N_i / \Sigma_i N_i = m + \sigma^2/m \quad (2)$$

where m is the mean and σ^2 the variance of the distribution of the number of new sexual partners per unit of time⁸. Thus, c is not simply the mean but the mean plus the ratio of variance to mean, which reflects the disproportionate role played by highly active individuals (in the tail of the probability distribution of sexual activity), who are both more likely to acquire infection and more likely to transmit it. The basic reproductive rate for HIV infection, R_0 , is related to the parameters β , c and D , and hence to Λ by the formula

$$R_0 = \beta c D \quad (3)$$

In contrast with standard epidemiological models in homogeneous populations (where the exponential phase of rising incidence lasts until something like half the pool of susceptibles have been infected), the early exponential phase is of

relatively short duration in our HIV models, giving way to a more nearly linear rise in the fraction infected (see Fig. 2).

This is because most susceptibles in the sexually highly active categories are infected in the early stages of the epidemic, producing saturation effects in these categories which decrease the exponential rise in incidence within them; although the incidence of infection continues to rise among individuals in less sexually active categories, the overall rate of increase is now slower than exponential.

Much less information is available about the rise in the number of individuals infected with HIV, as a function of time, than about the rise in the subsequent incidence of AIDS^{46,12,13}, largely because information about infection requires serological examination for antibodies to the HIV virus. Although the initial infection may produce symptoms^{3,16,17} and, in some cases acute encephalopathy¹⁸ and meningitis¹⁶, it is not clear that such symptoms are always evoked: in any event, the symptoms are usually sufficiently mild to preclude systematic reporting. By contrast, the opportunistic infections cancers^{4,5,12,19}, and subsequent mortality characteristic of the destruction of the immune system in AIDS, leads to fairly reliable reporting²⁰. There is, however, one study of hepatitis B virus (HBV) in a cohort of 6,875 homosexual and bisexual males in San Francisco, which resulted in serum samples being taken and preserved as early as 1978^{12,21,22}; stored sera of a representative sample of 785 of these individuals gives the rise in the fraction seropositive for HIV, from 1978 to 1985,

Table 1 Doubling time of the HIV epidemic (in the early stages)

Area	Serological data Period	Doubling time t_d (in months)
(a) Male homosexuals		
San Francisco, USA	1978 – 80	10 – 11
New York City, USA	1979 – 80	10 – 11
London, UK	1982 – 84	9 – 10
(b) Intravenous drug users		
Italy	1980 – 83	15 – 16
London, UK	1983 – 85	11 – 12
Switzerland	1983 – 84	8 – 9
Case notifications		
(a) All risk groups		
Australia	1982 – 85	4 – 5
Austria	1983 – 85	15 – 16
Belgium	1982 – 84	11 – 12
Canada	1981 – 85	9 – 10
Denmark	1982 – 84	13 – 14
Europe (EC)	1982 – 84	8 – 9
France	1982 – 84	8 – 9
Italy	1982 – 84	7 – 8
Netherlands	1982 – 84	7 – 8
Spain	1982 – 84	6 – 7
Sweden	1983 – 85	8 – 9
Switzerland	1983 – 85	9 – 10
United Kingdom	1982 – 84	10 – 11
United States	1982 – 83	5 – 6
West Germany	1982 – 84	6 – 7
(b) Heterosexuals		
United States	1982 – 84	9 – 10
Average		9 – 10

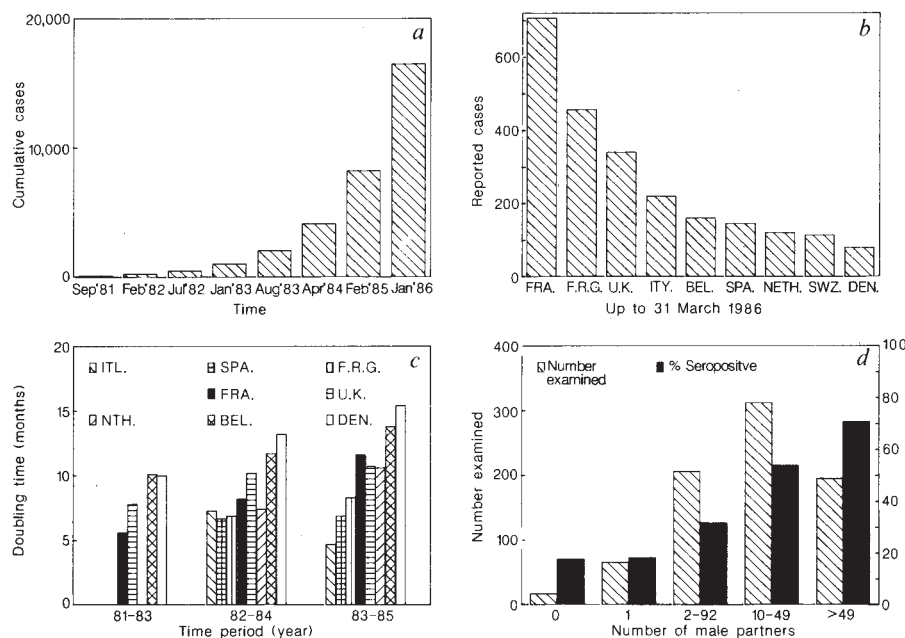


Fig. 2 *a*, The rise in the cumulative number of reported cases of AIDS in the USA over the interval September 1981 – January 1986¹³. *b*, Reported cases of AIDS in 9 countries of the European Community up to 31 March 1986⁴⁰. *c*, Doubling times in the cumulative incidence of AIDS (t_d) recorded in months for various European countries⁴⁰ over various time intervals (1981–83, 1982–84, 1983–85; DEN, Denmark; BEL, Belgium; NTH, Netherlands; FRA, France; E.C., European Community in total; F.R.G., Federal Republic of Germany; SPA, Spain; ITL, Italy; UK, United Kingdom). *d*, The relationship between sexual activity amongst a sample of homosexual/bisexual males (from San Francisco, USA) as measured by the number of male partners over a two-year period, and the percentage of each group (based on sex partners) who were seropositive for HIV antibodies (data from ref. 26).

shown in Fig. 1.

The pattern of roughly linear rise shown in Fig. 1 is uncharacteristic of standard epidemics (in homogeneously mixed populations), but is suggested by our HIV models. In Britain and other countries in Europe, the virus seems first to have appeared several years later than in the United States (Fig. 2*a*, *b* and *c*), and the spread of infection is still in its early stages. As a result there are serological studies focused on HIV roughly from its initial appearance in Europe^{23,24} (Fig. 1). The initially exponential rise in HIV infection may be characterized by a doubling time, t_d , related to the growth rate, Λ , of (1) by $t_d = (\ln 2)/\Lambda$.

Table 1 summarizes information about doubling times deduced from serological and case notification studies, which lead to a surprisingly consistent estimate of $t_d \sim 8$ –10 months in the early stages of the epidemic (Fig. 2*d*) giving an estimate of Λ of about 1.0 yr^{-1} . The characteristic duration of infection (and infectiousness), D , is probably not significantly less than the characteristic time from HIV infection to manifestation of AIDS.

But D may be significantly longer if a substantial proportion of infected individuals remain asymptomatic carriers (with the epidemiology similar to hepatitis B virus²⁵). On the other hand, recent studies observing that measurable HIV antigen (HIV-Ag, the presence of which indicates

the presence of the virus) appears early and transiently in primary HIV infection, that antibody production follows (1–3 months after infection) and that HIV-Ag may then disappear could imply lower estimates of D , as would the apparent correlation of this persistence or reappearance of antigen with clinical, immunological and neurological deterioration³.

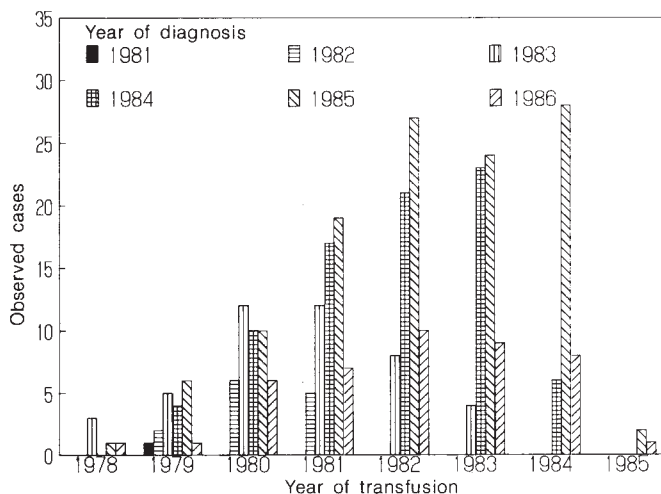
In the absence of conclusive data on infectiousness during the incubation period, we shall assume that D is equal to the incubation period. Studies of cases of AIDS associated with transfusion suggest that the average incubation period is 4–5 years³¹, but as such studies are extended, this estimate will rise (Fig. 3). The true average may be 8–10 years or more. Our estimate of Λ in conjunction with equation 1 then leads to the rough estimate

$$\beta c \approx 1 \text{ yr}^{-1} \quad (4)$$

Note that $\Lambda = \beta c$ provided D is large (4–5 years plus). Thus data on changes in seropositivity over time have allowed us to infer the approximate magnitude of the combination of epidemiological parameters β and c , neither of which can easily be estimated directly.

Is this estimate consistent with what is known about β and c separately? Unfortunately, nearly all the information about degrees of sexual activity among male homosexuals has focused on average numbers of sexual partners, as distinct

Fig. 3 Data on the distribution of the incubation period of AIDS derived from longitudinal studies of transfusion recipients (data from ref. 31). Observed cases of AIDS are recorded as a function of the year of transfusion (the assumed point of acquisition of infection) and the year of diagnosis. A Weibull distribution provides a good empirical description of this data with a mean incubation period of ~ 4 –5 years.



from average number of new partners per unit time^{26,27} (Fig. 4). For less active individuals (say, 1–3 partners per 6-month interval), the rate of acquisition of new partners will be seriously overestimated by the average number of partners. On the other hand, the quantity c is disproportionately influenced by highly active individuals, most of whose partners are likely to be new, so that studies based simply on numbers of partners may give a rough guide to the magnitude of c (Fig. 4). Quantitative information on average

values of β , whether for homosexuals or heterosexuals, is very limited at present. Estimates vary widely (from 0.05 to 0.5) although it appears that the average probability of transmission per partner contact is higher among male homosexuals than among heterosexuals, perhaps as a result of more frequent sexual activity that results in epithelial damage (for example anal intercourse)^{26,29}. Our estimates of $\beta c \sim 1 \text{ yr}^{-1}$ together with the high estimates of c for homosexuals suggest that β may be small (~ 0.05). But estimates of c based on

the reported number of partners per unit of time may significantly overestimate the number of new partners per unit of time, which, or that equating D to the incubation period, may overestimate the average duration of infectiousness. It may also be that the high values of c arise from sampling biased towards the high activity groups of homosexual communities.

As public awareness about AIDS has increased, there have been changes in patterns of sexual activity among male homosexuals in the United States (reflected, for example, in marked decreases in the incidence of rectal gonorrhoea^{29,30}) which have presumably resulted in changes both in β and in c ²⁷. Our discussion, therefore, pertains mainly to the relatively early stages — 1978 to the early 1980s — of the epidemic.

Incubation period

Although much more information is available about the incidence of AIDS than of HIV infection (Table 1), it is harder to tease estimates of epidemiological parameters out of these. Incidence of AIDS depends not only on the transmission factors β and c , but also on the incubation period and on the fraction, f , of those infected who will eventually develop AIDS.

Significantly, estimates of both the incubation period and f have tended systematically to increase since the epidemic was first recognized^{4,5,28,31,32}. Estimates of f range from 10% to 75% or more^{19,28,33,37}, with an incubation period of 4–5 years or more³¹. The progressive sequence of steps which eventually impair the ability of the immune system to respond to opportunistic infection seem not to be reversible. But whether all those infected with HIV are moving toward AIDS at different rates, or whether some will develop AIDS while others never will, remains unclear. Variability in the incubation period, and whether or not an infected person develops AIDS, could be accounted for by genetic heterogeneity within the host population (HLA-linked^{38,39}), or could be associated with specific strains of the antigenically variable HIV virus^{1,6}.

Studies of the incubation period for those who develop AIDS suggest that the 'hazard function', the probability of the disease manifesting itself as a function of the time since infection, increases with time (Fig. 3). Lui and co-workers³¹ have assumed a Weibull distribution (a flexible two parameter probability distribution) for the incubation period with probability density function

$$h(t) = \gamma v^{-\gamma} t^{\gamma-1} \exp[-(t/v)^{\gamma}] \quad (5)$$

If indeed the probability per unit time, to develop AIDS (for that fraction f who do indeed develop it) increases linearly with time from infection as αt , the result is a Weibull distribution with $\gamma = 2$ and $v = \alpha$ for the hazard function⁸. This

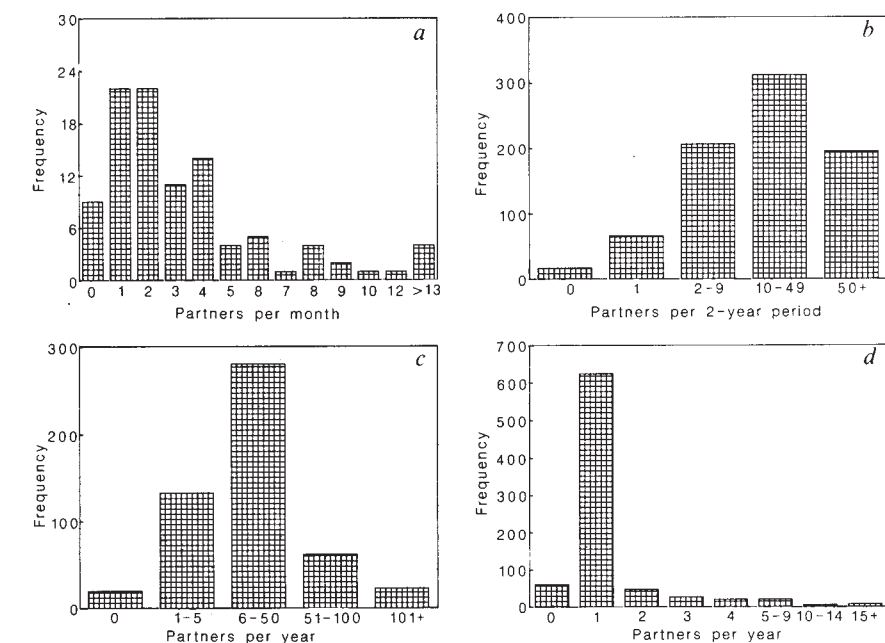
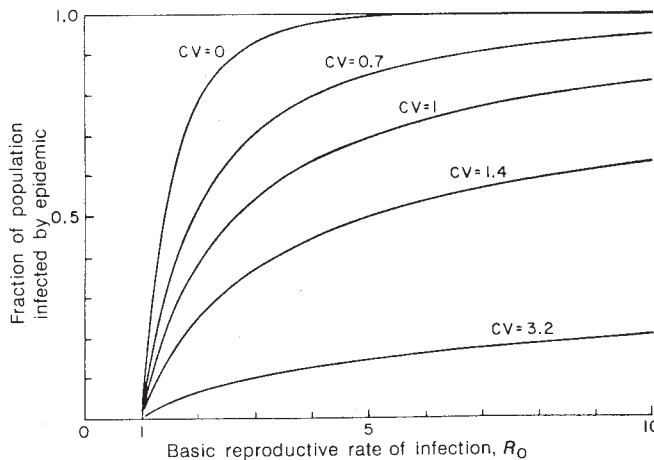


Fig. 4 Studies of sexual activity amongst *a*–*c*, male homosexual and *d* heterosexual communities. The graphs record frequency distributions of the number of sex partners per defined time period in samples of homosexual/bisexual males and heterosexuals. *a*, Homosexual/bisexual males resident in London surveyed in 1986 (unpublished data from C.A. Carne and I.V. Weller) ($m = 4.7$ per month, $\sigma^2 = 56.7$). Data denote male partners per month. *b*, Homosexual/bisexual males resident in San Francisco surveyed in 1984–85 (data from ref. 26). Data denote male partners per 2-year period. *c*, Homosexual/bisexual males resident in London surveyed in 1984 (unpublished data from T. McManus). Data denote male partners per year. *d*, Heterosexuals between the ages of 18–44 years in England surveyed in November 1986 (unpublished data, Harris Research Organisation; R.M.A. and G.F. Medley). Data denote partners of the opposite sex per 1 year period (sample size = 823, $m = 1.41$, $\sigma^2 = 4.36$). A further survey of homosexual men (ref. 27) in San Francisco reveals a decline in the mean partners per month over the period November 1982 to November 1984 from 5.9 to 2.5.

Fig. 5 The relationship between the eventual fraction infected (seropositive) in an epidemic of HIV and the basic reproductive rate R_0 (see text). The predictions are based on a model which assumes that sexual activity (defined as the number of new partners per unit of time) obeys a gamma distribution with varying coefficients of variation (CV) (mean m and variance σ^2 ; see text).



assumption differs from conventional epidemiological models, where infected individuals move through the incubation interval either at a fixed rate, or in a fixed time. But none of this resolves the question of what proportion of those infected will develop AIDS on what timescale: That issue will be resolved only by very long term (many decades) studies.

Fraction eventually infected

In a closed and homogeneously mixed population, the total fraction eventually infected depends only on the basic reproductive rate of the infection, R_0 , defined above as shown⁷ by the uppermost curve in Fig. 5. For sexually-transmitted infections such as HIV, the result can be extended to include the complications associated with a wide diversity in degrees of sexual activity.

In a closed population, the eventual fraction seropositive will depend both on R_0 and on the actual distribution of rates of acquisition of sexual partners. Assuming a gamma distribution⁸, we may characterize it by c and by its coefficient of variation ($CV = \sigma/m$). The resulting overall fraction infected is shown as a function of R_0 , for a range of values of CV , in Fig. 5; for fixed R_0 , the eventual seropositive fraction can be much lower than for $CV = 0$, if the variability in degrees of sexual activity (measured by CV) is high. This makes intuitive sense: the highly active individuals acquire infection, and eventually are removed, relatively early in the epidemic; transmission among the remaining, less active, individuals may be relatively weak.

Figure 5 may be used, in combination with two factual observations, to make a rough assessment of R_0 for HIV among male homosexuals. First, the studies indicate great variability in degrees of sexual activity among male homosexuals (with CV significantly in excess of unity^{8,26,27}), thus confining attention to the lower curves. The second observation is that levels of seropositivity to HIV among male homosexuals in San Francisco in

1985 are variously reported as 70% or more¹² (in the HBV study which is probably biased towards more active individuals) and as around 50% (in a study carefully constructed to avoid bias²⁶), providing a lower bound of 50–70% on the proportion ever seropositive. For CV noticeably in excess of unity, this can be achieved only if R_0 is in excess of 5.

Thus our early estimate of $\beta c \sim 1 \text{ yr}^{-1}$, in conjunction with the assessment that R_0 exceeds 5, leads to an indirect estimate that D exceeds 5 years. Although R_0 , like β and c changes with changing social and sexual habits, the data leading to our earlier estimate for βc come from the early stages of the rise in HIV infection, before such changes were significant. The estimate of R_0 depends importantly on observed levels of seropositivity, but these were also high before social changes became pronounced. Consequently, our estimate of D which depends only on the basic biology of HIV, is reasonably consistent. This independent estimate of $D \sim 5$ years accords with current estimates that the incubation period is 4–5 years or more.

An estimate of the value of R_0 in the early stages of the epidemic is also valuable in indicating the magnitude of the social changes needed to bring R_0 below unity. If R_0 is around 5–10 or more, then reductions by a factor of 5–10 or more in βc are needed. Because c depends disproportionately on those in the highly sexually active category, programmes aimed at getting them to change their habits — both to fewer partners and to “safe sex” — are most efficient. But if such individuals are less likely to respond to public health education, it will be harder to bring R_0 below unity.

Mortality

The frequent assumption that the severity of the epidemic, in terms of cumulative mortality, will be greatest if all those infected eventually develop AIDS and subsequently die is not necessarily true. Mortality depends critically on the duration of infectiousness of both those in-

fectured who develop AIDS and those infected who do not. If the latter have a similar life expectancy to those not infected, but remain infectious for life, they may contribute more to the net transmission of the virus, R_0 , than those who die of AIDS. Much may be understood by recognizing that the overall net reproductive rate of the virus, R_0 , is made up of two components, the reproductive rate of those who develop AIDS (R_{01}) and the equivalent rate of those who do not (R_{02}). If a fraction f develop AIDS

$$R_0 = fR_{01} + (1-f)R_{02} \quad (6)$$

where the two reproductive rates are defined by equation (3) with different parameters for the separate groups. Even if the asymptomatic carriers are less infectious than those who develop AIDS, if they remain infectious over, say, a 30-year span of sexual activity, R_{02} may be much larger than R_{01} , and, depending on f , the contribution of the asymptomatic carriers to R_0 may be dominant.

At present, it is not possible to tell whether the severity of the epidemic will be increased or decreased if a larger fraction of those infected develop AIDS, for the relative infectiousness of the two categories is unknown. For public health planning it is clearly important to attempt to acquire such data.

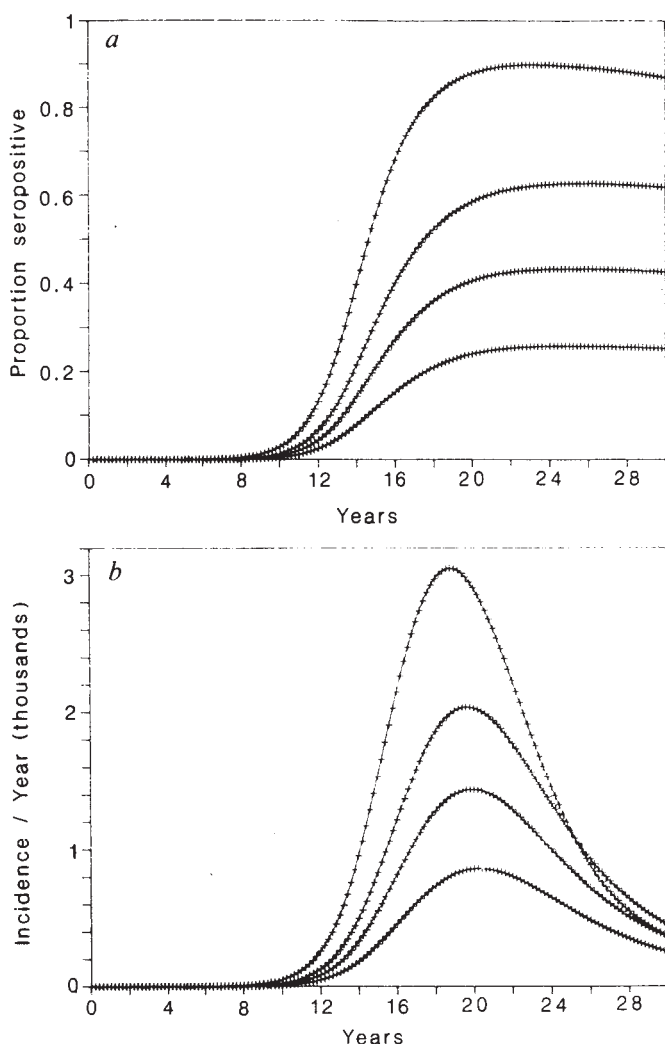
Dynamics of the epidemic

The dynamics of an HIV epidemic within a homosexual community are represented by the results of our calculations given in Fig. 6, which shows the proportion seropositive and the incidence of cases of AIDS as a function of time since the start of the epidemic. It is assumed that 30% of those infected eventually manifest AIDS, with the incubation intervals obeying a Weibull distribution such that the average incubation period is 5 years³¹. Individuals who are incubating AIDS are assumed infectious throughout the incubation interval, and the 70% who remain asymptomatic are assumed to remain infectious for similar periods.

Many of the features presented in Fig. 6 show qualitative agreement with observation. The rise in incidence of infection (seropositivity) is initially exponential, but soon shows a more linear rise. And, the rise in incidence of AIDS lags that in the proportion infected, as seen.

It is easy to build epidemiological models of arbitrary complexity, which may appear beguilingly realistic, but we think there is little point in constructing them until more is known about the relevant epidemiological parameters. We distrust predictions made by using statistical procedures to fit polynomial or exponential curves to existing data on the incidence of AIDS, and then extrapolating^{40,44}. The HIV epidemic is a dynamic process; to predict future trends, models

Fig. 6 The predictions of a model (see ref. 8) incorporating variable incubation periods, heterogeneity in sexual activity and recruitment of susceptibles. The two graphs record changes in seropositivity through time from the point of introduction of HIV into a community of 100,000 homosexual/bisexual males (graph *a*) and the incidence of AIDS yr^{-1} (graph *b*). Heterogeneity in sexual activity is described by a gamma distribution with a mean fixed at 5 partners yr^{-1} and variances 5, 25, 50 and 100 representing the predictions recorded by the four lines depicted in each graph. In *a* and *b* the smallest epidemic arises when the variance is largest and vice versa. Parameter values, $R_0 = 5$, $D = 5 \text{ yr}$, $f = 0.3$ with the life expectancy of AIDS patient set at 1 yr from diagnosis and for the susceptible sexually active community at 32 yr from the point of joining the sexually active class. The 70% of infected who do not develop AIDS are assumed to be infectious for a period equal to D . The immigration of new susceptibles into the sexually active community was set at 100,000 per 32 yr.



must be based on the underlying epidemiological phenomena.

Heterosexual transmission

In developed countries, the extent to which HIV infection can be transmitted by heterosexual contacts is uncertain^{43,48}. HIV infections in females come from contact with bisexual males (the dominant sexually-transmitted route at present), transfusion recipients, haemophiliacs and intravenous drug users⁴⁵. If such females are not themselves a significant source of infection back into the homosexual/bisexual community (through contacts with uninfected bisexuals), we would expect the incidence of HIV infections among the female partners of bisexuals initially to rise roughly in proportion to the incidence among homosexual males.

Specifically, we would expect the ratio of HIV infection among female partners of bisexuals to that among bisexual males to be $\sim \beta'c'/\beta c$, where β and c are as previously defined, β' is the transmission probability for male-to-female contact, and c' is the mean number of new female partners acquired by a bisexual male, per unit time. We expect this ratio to be sig-

nificantly less than unity, because β' is less than β , and c' significantly less than c . The data (Table 1) suggest the doubling time for heterosexually-transmitted HIV infection is roughly equal to that among homosexual males, which is in accord with our simple expectation.

As the epidemic progresses, a high proportion of homosexual males become infected (Fig. 2e). On the other hand, the pool of female partners for bisexual males may be large, and we would expect incidence of HIV infection still to be rising roughly linearly among women at and beyond the point at which saturation effects limit the epidemic among homosexual/bisexual males. How long this rise continues, and how many females are eventually likely to be infected, depends on the average duration of infectiousness in the transmitting group of males (which could be long if a substantial fraction remain asymptomatic carriers).

The transmission of HIV infection to females by bisexual males is a process whose initial dynamics is essentially determined by R_0 for transmission among homosexual males, thereafter the ques-

tion of its transmission and maintenance by purely heterosexual contact arises. The basic reproductive rate for such heterosexual transmission of HIV, R_0' , is given by

$$R_0' = (\beta_1\beta_2c_1c_2)^{1/2}D \quad (7)$$

Here β_1 and β_2 are the transmission parameters for contacts between infected females and susceptible males and between infected males and susceptible females, respectively; c_1 and c_2 are as before given by (2), for the distribution in rates of acquiring new partners of the other sex by females and males, respectively.

Data are very limited on the transmission and sexual activity parameters, but the data in Fig. 4 suggest that c_1 and c_2 are significantly smaller than c among homosexual males. Further, it seems likely that $\beta_1 < \beta_2$ and that both are less than the MM for homosexual males. Thus overall, the factor $(\beta_1\beta_2c_1c_2)^{1/2}$ seems likely to be much smaller than c for homosexual males, which suggests that in developed countries, R_0 for purely heterosexual transmission is probably significantly smaller than R_0 for purely male homosexual transmission. Whether R_0 is greater than unity, such that HIV infection can maintain itself and spread by purely heterosexual transmission, is at present unclear. There is an urgent need for studies to measure c_1 and c_2 in different communities (stratified by age and social status) and to assess how these parameters change as a consequence of educational programmes and publicity campaigns on AIDS. The use of professional opinion poll organizations to gather quantitative data on rates of partner change over a series of specified time intervals by interview and questionnaire (Fig. 4d) could help to fill this gap in our knowledge, but estimates of β_1 and β_2 will come only from long term studies of the heterosexual partners of infected patients.

If R_0' does exceed unity, the incidence of HIV infection in the heterosexual community will initially grow exponentially, at a rate given by the analogue of equation (1):

$$\Lambda = (\beta_1\beta_2c_1c_2)^{1/2} - 1/D = (R_0' - 1)D \quad (8)$$

The estimates above indicate that initial doubling times will be significantly longer than the 9 months or so for HIV among homosexual males; the slow initial growth will be difficult to discern against a background of homosexual transmission among males and bisexual transmission to females.

These observations are not necessarily inconsistent with the epidemiological situation for HIV in sub-Saharan Africa^{6,49,56,57}. In contrast to the United States and the United Kingdom, where male/female ratios of AIDS cases have been of the order of 14:1 to 20:1, in certain parts of central Africa including areas in Zaire, Rwanda and Uganda, sex ratios approaching unity have been reported^{45,50-53,56,57}. Very high prevalences of HIV antibodies have been found in males and

females from surveys in urban and rural areas^{52,56,57}. These points suggest that heterosexual transmission has been frequent in both directions and horizontal studies have shown that infection is associated with the age-related degree of sexual activity amongst heterosexuals^{27,47,48,56,57}. We note, however, that in the early and approximately exponential phase of the epidemic, the ratio of the number of seropositive males to seropositive females is not unity, but is roughly $(\beta_1 c_1 / \beta_2 c_2)^{1/2}$.

It is generally thought that β_1 is less than β_2 for HIV, although the facts are uncertain (for gonorrhea, for instance, male-to-female transmission, β_2 is roughly twice β_1). Obviously the average number of heterosexual partners of females and males, m_1 and m_2 , are equal, but c_1 could significantly exceed c_2 if the variance of the distribution of rate of acquiring new sexual partners by females (associated with the concentrated activities of female prostitutes) is greater than that for males. This effect could partly offset β_1 being smaller than β_2 . Although there is no *a priori* reason to expect the ratio $\beta_1 c_1 / \beta_2 c_2$ to be exactly unity, its square root could easily be close to unity, which would explain the roughly equal proportions of seropositive males and females. Alternatively, the roughly equal proportions could be explained if homosexual transmission among males had coincidentally raised the seropositive proportion among males to around the level among females, or by transmission by contaminated needles in public and private medical services⁶. In any event, the rough equality of the seropositive proportions among males and females is a puzzle to be explained, and is not by itself evidence for purely heterosexual transmission

Discussion

The ideas presented above are based on relatively simple mathematical models, with the aim of making clear some of the essential relations between epidemiological parameters and the overall course of HIV infection within various populations. Such models help to clarify what kinds of epidemiological data are needed to make predictions. As such data become available, the models can be made more detailed and realistic.

For public health planning, the dominant unknown is f , the fraction infected who will eventually develop AIDS. Estimates of this parameter have been increasing in recent years, but on present evidence the possibility cannot be ruled out that it is as low as 20% or as high as virtually 100%. Thus any current predictions about the number of homosexuals likely to acquire AIDS are uncertain by at least a factor 5 or so. Better understanding of the mechanisms of interactions between virus and host may help to determine f , but it is possible that only

epidemiological data gathered on a decade-long timescale, as cases accumulate, will resolve this question.

The duration of infectiousness, and the way this duration is distributed among different infectives, is also relevant to estimates of R_0 and thence of the eventual number infected; more studies directed towards eliciting this information, including looking for virus in the blood, excretions and secretions of infected individuals over time, together with longitudinal studies of the partners of infected patients, are needed³.

More generally, there is need for more studies that combine information about the epidemiological history of individuals with information about their sexual habits, such as the important study by Winkelstein *et al.*²⁶ of an unbiased sample of homosexuals in San Francisco, which demonstrated the association between the number of sexual partners and probability of acquiring infection. We emphasize that what is epidemiologically important is the average rate of acquiring new sexual partners, not necessarily the same as the average number of partners per unit time. Some authors have recognized that sexually highly active individuals play a disproportionate role in the transmission dynamics; equation (2) quantifies this observation, making it clear that the epidemiologically relevant quantity is not the mean number of new partners but, rather, the mean-square divided by the mean.

In developed countries, at present and into the near future, it is probable that sexually-transmitted HIV infections among females are likely to come mainly from bisexual males. Whether subsequent spread of infection from such females to heterosexual male partners is likely to reach significant levels, and more im-

portantly whether purely heterosexual transmission of HIV infection may be self-sustaining ($R_0' > 1$), depends on estimates of the transmission parameters β_1 , β_2 , c_1 and c_2 .

We have shown how c for transmission among homosexual males can be estimated indirectly from data on initial doubling times, but corresponding estimates of $\beta_1 c_1$ and $\beta_2 c_2$ are much harder, partly because the corresponding doubling rates are likely to be longer and partly because these infections are likely to be masked by homosexual/bisexual transmission among males, and by bisexual-to-female transmission among females (both of which processes depend simply on c). Attempts to estimate these quantities directly, and thence to estimate R_0 , are urgently needed.

From present knowledge, it is not possible to assess whether R_0' is greater or less than unity in developed countries, and it is thus not possible to say whether HIV infections could spread epidemically by purely heterosexual transmission. The evidence from Africa, however, clearly argues that the sexually active population as a whole should be regarded as at risk^{6,47,56,57}.

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- Hahn B.H. *et al.* *Science* **232**, 1548-1552 (1986).
- Goedert, J.J. *et al.* *Lancet* **ii** 711-715 (1984).
- Goudsmit, J. *et al.* *Lancet* **ii** 177-180 (1986).
- Peterman, T.A., Drotman, D.P. & Curran, J.W. *Epidem. Rev.* **7**, 1-21 (1985).
- Curran, J.W. *Ann. Intern. Med.* **103**, 657-662 (1985).
- Acheson, E.D. *Lancet* **i** 662-676 (1986).
- Anderson, R.M. & May, R.M. *Nature* **318**, 323-329 (1985).
- Anderson, R.M., May, R.M., Medley, G.F. & Johnson, A. *IMA J. Math. Med. Biol.* (in the press).
- Dietz, K. *Lect. Notes. Biomaths.* **11**, 1-15. (1976).
- Anderson, R.M. in *Theoretical Ecology* (ed. May, R.M.) 318-355 (Blackwell, Oxford, 1981).
- Hethcote, H.W. & Yorke, J.A. *Lect. Notes. Biomaths.* **56**, 1-105 (1984).
- Centers for Disease Control MMWR **34**, 573-589 (1985).
- Centers for Disease Control MMWR **35**, 17-20 (1986).
- Anderson, R.M. & May, R.M. *Nature* **280**, 361-367 (1979).
- May, R.M. & Anderson, R.M. *Nature* **280**, 455-461 (1979).
- Ho, D.D. *et al.* *New Engl. J. Med.* **313**, 1606 (1985).
- Ho, D.D. *et al.* *Ann. Intern. Med.* **103**, 880-883 (1985).
- Carne, C.A. *et al.* *Lancet* **ii** 1206-1208 (1985).
- Wong-Staal, F. & Gallo, R.C. *Nature* **317**, 395-403 (1985).
- Centers for Disease Control MMWR **34**, 373-375 (1985).
- Schreeder, M.T. *et al.* *J. Infect. Dis.* **146**, 7-15 (1982).
- Jaffe, H.W. *et al.* *Ann. Intern. Med.* **103**, 210-214 (1985).
- Carne, C.A. *et al.* *Lancet* **i**, 1261-1262 (1985).
- Angarano, G. *et al.* *Lancet* **ii** 1302 (1985).
- Francis, D.P. *Rev. Infect. Dis.* **5**, 322-329 (1983).
- Winkelstein, W. *et al.* *J. Am. Med. Assoc.* **257**, 321-325 (1987).
- McKusick, L. *et al.*, *Pub. Hlth. Repts.* **100**, 622-628 (1985).
- Curran, J.W., Morgan, W.M. & Hardy, A.M. *Science* **229**, 1352-1357 (1985).
- Weller, I.V.D., Hindley, D.J. & Adler, M.W. *Brit. med. J.* **289**, 1041 (1984).
- Centers for Disease Control MMWR **34**, 613-615 (1985).
- Lui, K.J. *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **83**, 3051-3055 (1986).
- Peterman, T.A. *et al.*, *J. Am. med. Ass.* **254**, 2913-2917 (1985).
- Moss, A.R. *et al.*, *J. infect. Dis.* **152**, 152-161 (1985).
- Weber, J.N. *et al.*, *Lancet* **ii** 1179-1182 (1986).
- Stevens, C.E. *et al.*, *J. Am. med. Assoc.* **255**, 2167-2171 (1985).
- Goedert, J.J. *et al.*, *Science* **231**, 992-995 (1986).
- Brodt, H.R. *et al.* *Dtsch. med. Wschr.* **111**, 1175-1180 (1986).
- Weiss, R.A. in *Virus Resistance* (eds Mahy, B.W.J., Mison, A.C. & Dorby, G.K.) 267-288 (Cambridge Univ., London, 1982).
- Scorza Smeraldi, R. *et al.* *Lancet* **ii** 1187-1189 (1986).
- McEvoy, M. & Tillett, H. *Lancet* **ii**, 541-542 (1985).
- Artalejo, F.R. *et al.*, *Lancet* **i** 378 (1986).
- Downs, A.M., Ancelle, R. & Brunet, J.B. (in the press).
- Barnes, D.E. *Science* **232**, 1589-1590 (1986).
- Gonzalez, J.J. & Keoch, M.G. *W.H.O. Report on Euro meeting on AIDS containment* (WHO, Geneva, 1986).
- Vogt, M.W. *et al.*, *Lancet* **i**, 525-527 (1986).
- Wofsy, C. *et al.*, *Lancet* **i**, 527-529 (1986).
- Centers for Disease Control MMWR **34**, 561-563 (1985).
- de Perre, P.V. *et al.*, *Lancet* **ii** 524.
- Harris, C. *et al.*, *New Engl. J. Med.* **308**, 1181-1184 (1985).
- Centers for Disease Control MMWR **31**, 697-698 (1984).
- Newmark, P. *Nature* **322**, 6 (1986).
- Biggar, R.J. *Lancet* **i** 79-82 (1986).
- Kreiss, J.K. *et al.*, *New Engl. J. Med.* **314**, 414-417 (1985).
- Papervangelou, G., Roumeliotou-Karayannis, A., Kallinikos, G. & Papoutsakis, G. *Lancet* **ii** 1018 (1985).
- Tirelli, U. *et al.*, *Lancet* **ii** 1424 (1985).
- Quinn, T.C., Mann, J.M., Curran, J.W. & Piot, P. *Science* **234**, 955-963 (1986).
- Melbye, M. *et al.* *Lancet* **ii** 1113-1117 (1986).

Late Cretaceous and paroxysmal Cretaceous/Tertiary extinctions

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The various geological signatures at Cretaceous/Tertiary time including iridium and other associated elements, microspherules, and shock deformation features are compatible with the suggestion that the transition is marked by a period of intense volcanism. The volatile emissions from this volcanism would lead to acid rain, reduction in the alkalinity and pH of the surface ocean, global atmospheric temperature changes, and ozone layer depletion. These environmental effects coupled with those related to the major sea level regression of the late Cretaceous provide the framework for an explanation of the selective nature of the observed extinction record.

THE transition from Cretaceous to Tertiary 65 million years ago marked one of the major extinction periods in Earth history. The geological record contains evidence of many events at this time; thus it is not surprising that there has been considerable controversy about the causes of the extinctions. Any explanation must take into consideration the selective nature and chronology of these extinctions as well as whatever geological evidence is available to support ideas about the causes of the environmental changes. A provocative model was developed by Alvarez *et al.*¹ in support of the impact of an extraterrestrial object as the cause, based primarily on an excess of iridium in the vicinity of the boundary. This was a key observation and the model has stirred a great deal of interest and research on the subject. Although in a later paper Alvarez *et al.*² conceded the possible role of other factors, attention has been largely focused on the impact idea in discussing the mass extinction event, because of the iridium anomaly, and accordingly we concentrate on this.

Other geological evidence is very limited and the crater from the proposed impact has proved to be elusive. Furthermore, even if the iridium were of extraterrestrial origin, the dust cloud mechanism originally suggested as a cause for the extinctions would be more appropriate for total and geologically instantaneous extinctions rather than the selective extinctions observed which in most cases are distributed over a geologically significant time period. Therefore, it is useful to examine alternative models if these are plausible in terms of the record of the rocks and are more selective in their effects on organisms.

We suggest that there is strong evidence that the ultimate causative agent was not extraterrestrial but intrinsic to this planet. The argument is put forward that superimposed upon major environmental changes bound up with eustasy and climate over a longer time interval, there were significant environmental perturbations related to increased volcanism across the Cretaceous/Tertiary (K/T) boundary. In the following we first review the record of the faunal and floral changes that occurred. We next review the geological evidence of increased volcanism at K/T time and examine the environmental effects that might be expected from the volcanic emanations. We then discuss the faunal and floral changes that would be expected to have occurred as a result.

We wish to emphasize that this article predominantly advocates a particular point of view and may be criticized in giving less attention to alternative models. The calculations for the environmental effects of intense global volcanism are order of magnitude determinations. The intent has been to obtain reasonable estimates of these effects and to assess to what degree they,

coupled with the anticipated effects of the Late Cretaceous sea level regression, may lead to an explanation for the difficult problem of both the selective nature of the K/T extinctions and the temporal sequence and intervals in which the extinctions occurred.

Fauna and flora at the end of the Cretaceous

The end of the Cretaceous is marked by the extinction of the marine reptiles, the flying reptiles, dinosaurs and ammonites together with numerous families of scleractinian corals, bivalves such as the inoceramids and rudists, gastropods and echinoids. In addition the coccolithophorids, planktonic foraminifera and belemnites suffered almost complete, though not geologically instantaneous, extinction with only a few species surviving the crisis; many genera of the larger benthic foraminifera and radiolaria also disappeared. On the other hand, and making due allowance for the poorer quality of the fossil record, a number of groups were little affected including many types of land plants, freshwater vertebrates, snakes, mammals and many marine invertebrates including deep sea benthic organisms³. However, a short-term perturbation at the species level at the horizon of the iridium anomaly seems to be recognizable locally in some invertebrate groups².

Extinction was particularly severe among planktonic organisms with calcareous skeletons, tropical reef invertebrates, and animals of large size in terrestrial communities. Tropical reef invertebrates were seriously affected but there are few data for the upper part of the Maastrichtian. Any extinction model should also take into account some further facts recognizable from the stratigraphic record. While the calcareous plankton extinctions apparently took place over a relatively short geological time interval of ~10,000 yr⁴ and perhaps as little as 200 yr in some cases⁵, other marine extinctions were more prolonged or took place earlier. Thus the ammonites had been in decline from the Campanian through the final, Maastrichtian, stage of the Cretaceous with the survivors of the decline being long-lived genera that had been relatively extinction resistant; the cause of the final extinction of these genera could well have been different from the cause of the more general late Cretaceous decline⁶⁻⁸. Although precise stratigraphic data are sparse, it appears that the most significant extinction event among shallow marine tropical benthic organisms such as rudists, hermatypic corals and large foraminifera was within the Maastrichtian, a few million years before the end of the Cretaceous⁹. As a consequence of late Cretaceous sea level regression, continuous shallow marine sections across the K/T boundary are rare

and as a result it is not yet possible to determine if terminal Cretaceous benthic extinctions were on a global scale and therefore comparable to the contemporary event in the plankton.

Magnetostratigraphic and radiometric correlations indicate that extinctions of marine and terrestrial organisms were broadly contemporaneous but the precision is no better than a few hundred thousand years, and within terrestrial stratigraphic sections the last appearance of the dinosaurs and the floral K/T boundary are not coincident¹⁰⁻¹⁴. However, there is clearly a degree of inference involved in equating 'highest occurrence' in a stratigraphic section with 'last appearance' on earth. Changes at the K/T boundary in the angiosperm floras were relatively minor and geographically variable on a worldwide scale; they were preceded by a decline in diversity through the Maastrichtian¹⁵. Recent studies indicate that although some Cretaceous floral taxa became extinct, others were minimally affected at the K/T boundary and reappear in strata above¹⁶.

Discussion of terminal Cretaceous extinctions has been dominated by the dinosaurs but Padian and Clemens¹⁷ have pointed out that the terminal Cretaceous event may be nothing extraordinary in terms of rate or magnitude of extinction as compared with many extinction events earlier in dinosaur history, which is marked by high generic turnover rates of the order of a few million years or less. The significant difference therefore appears to be a fall in origination rate with the disappearing genera failing to be replaced. Furthermore, the latest report on dinosaur extinction in North America¹⁸ suggests that it was a gradual affair which began 7 million years before the end of the Cretaceous and accelerated rapidly in the last few hundred thousand years during an interval of apparent competition from immigrant mammals. The last dinosaurs are claimed to be Palaeocene in age and not reworked from Maastrichtian strata, and they occur above the iridium anomaly horizon. Most groups of freshwater vertebrates (fish, amphibians, turtles, crocodiles) survived the K/T boundary relatively unscathed and among the terrestrial vertebrate faunas only the marsupial mammals were badly affected. Lizards were little affected and placental mammals were actually increasing in diversity before the dinosaurs became extinct.

Whatever was responsible for the extinction events that have been associated with the iridium anomaly, it appears to have been preceded by an increase in extinction rate among a number of groups spread over a period of up to several million years. Thus the impact extinction model is misleading in focusing attention solely on this event. Even for the paroxysmal K/T event in the marine stratigraphic record, there are evident complexities that have not yet been adequately discussed or evaluated.

In particular a model for the K/T extinctions should include processes that might in due course provide an explanation for the timing of various species extinction events within the K/T transition as well as the temporal duration over which a given event occurs. The specifics of timing within the K/T transition are not known in detail but it is known that variations exist¹⁹. For example, in the marine sedimentary record the change from Cretaceous to Tertiary nannofossils generally occurred over a measurable section interval of around 50–100 cm^{4,20} though allowance must be made for bioturbational smearing. Planktonic foraminifera and nannoplankton (coccolithophorids) show a different extinction-recovery pattern at the K/T boundary. Whereas both groups are considerably reduced at the level of the iridium anomaly, the nannoplankton experienced final extinction well into the Tertiary^{4,20,21}. The lack of precise correspondence between the foram and coccolith extinctions implies a more complex scenario than has been widely assumed.

Geological signatures at K/T boundary

Six years ago the interest of the geoscience community was stimulated by the discovery of an enhancement of iridium and

other elements in clay layers at the K/T boundary in Italy and Denmark¹. The iridium was interpreted to be of extraterrestrial origin from an asteroid impact, an instantaneous event in geological terms, and the clay layer to be the global fallout of impact generated fine dust which had been injected into the stratosphere. Subsequent studies negate both interpretations. There is no ubiquitous K/T clay layer. At the Danish section the clay layer (Fish clay) is of shallow water origin containing fish remains and showing strong dissolution features²². At the Italian section the clay layer is one of many clay layers in an alternating sequence of limestones and thin clay interbeds extending from Turonian to Eocene²³. For most of the Deep Sea Drilling Project (DSDP) sections the K/T lithology is that of either a continuous calcareous sequence or a continuous pelagic clay sequence¹⁹. In Denmark the K/T Fish clay is smectite rich while in Italy the clay layers over a 3 m zone straddling the K/T boundary are enriched in kaolinite^{24,25}. In the Caucasus the clay is predominantly palygorskite²⁶; the clay mineralogy of the iridium-rich layer does not appear to be anomalous.

More recent and extensive iridium measurements show that the anomaly does not appear as a single spike in the record, indicative of an instantaneous event, but rather occurs over a measurable geologic time interval of 10 to 100 kyr or possibly longer²⁷. For Italy there is a peak in the iridium anomaly for the K/T clay as well as enhanced level of iridium above background in the other clay layers extending over 1–2 m (100–200 kyr) both above and below the reference K/T clay as well as in the intervening limestones²⁸. In Denmark similar results are found with enhanced levels of iridium extending into both the overlying Danian limestone and the underlying Maastrichtian chalk as well as in the Fish clay itself²⁹. For Red Deer valley, Canada, the iridium flux interval is estimated to be 5–6 kyr³⁰. All the DSDP sections that have been investigated also show an extended distribution of iridium at elevated levels of around 30 cm (30 kyr) at K/T time^{31–34}. The most recent iridium investigations presented at the 1986 annual meeting of the Geological Society of America show multiple iridium anomalies for the K/T sections at Bragg, Alabama³⁵, Brazos river, Texas³⁶ and Haiti³⁷.

We suggest that these observations are best explained in terms of intense global volcanic activity over 10 to 100 kyr at K/T time. The extended iridium distribution is difficult to explain in terms of an instantaneous event either of extraterrestrial or terrestrial origin, and iridium enhancements are known to be associated with airborne particles from recent hot spot/mantle plume volcanic activity³⁸. Further, the enhancements of other elements associated with the iridium anomalies are not in accord with an extraterrestrial origin; in particular the As/Ir and Sb/Ir ratios are three orders of magnitude greater than chondritic values but are in accord with a mantle origin²⁷.

There are other features associated with the K/T boundary. Microspherules are present. Some are of a K-feldspar composition, and these were originally interpreted to be high temperature droplets formed by an impact³⁹. The K-feldspar has now been interpreted as a secondary, diagenetic replacement product⁴⁰, and microspherules of different chemical compositions are found at various K/T sections^{41–44}. In Italy spherules were originally reported for numerous clay layers^{45,46}. Some of the spherules, however, for one of the Italian sections (Bottaccione) are contaminant hollow spherules of a recent organic origin⁴⁷. More recent analyses show that the solid spherules of K-feldspar or K-feldspar and glauconite composition for the Bottaccione section as well as for the Contessa section, which has no contaminant hollow spherules, have a concentration peak for the K/T clay as well as observable concentrations for other clay layers within 1–2 m above and below the reference K/T clay⁴³. Microspherules of clearly volcanic origin are known to occur^{48,49}. The volcanic microspherules are in the size range of 20–170 μm ^{48,49}; the K/T spherules studied at the Bottaccione and Contessa sections are in the size range 100–200 μm ⁴³. The

present size of the K/T diagenetic replacement spherules may or may not represent their original size.

Shock deformation features in quartz have been found at a palynological K/T boundary in the western United States^{50,51} and Canada⁵² as well as 25 cm below this boundary at one location⁵¹. They have also been found at a K/T section in Italy⁵³ and in Austria⁵⁴. Since shock metamorphic features are characteristic of materials associated with meteor craters⁵⁵, this finding was taken as supporting evidence for an impact origin for the K/T boundary events. A suggested candidate is the nearby Manson structure in Iowa⁵⁶, but this feature is apparently too small to account for the global K/T extinction events. On the other hand, if the quartz grains were transported out of the atmosphere and distributed around the world, as suggested in the dust cloud model, they would be expected to be annealed on their return to Earth, just as tektites are melted, and the shock features would not be retained. Shock features are also associated with the Vredefort intrusive and are interpreted to be of internal and magmatic origin there⁵⁷⁻⁵⁹. Recent studies have found shock metamorphic features in samples from the Toba caldera⁶⁰, Toba ash samples from Indian ocean deep sea cores⁶¹, and Bishop tuff ash samples from the Long Valley caldera eruption⁶². Toba is the largest known volcanic eruption of the recent past, occurring about 75,000 years ago with a total eruption magnitude of about 400 times that of Krakatoa⁶³. The eruption of Long Valley took place 600,000 yr ago producing pumice distributed throughout much of the western United States⁶⁴. The indicated peak shock stresses for explosive volcanism could account for the microstructures observed at K/T time and a volcanic origin must be considered for them.

Several investigators have noted that the period of the late Cretaceous to early Tertiary also coincides with the emplacement of the bulk of the Deccan Traps. They are located in western India and are the largest continental flood basalt deposits of the past 200 Myr with an estimated volume of $1 \times 10^6 \text{ km}^3$ (ref. 65). This number is probably on the low side as an estimate of their original volume for it does not include provision for post-emplacement erosion nor their possible offshore extension to the west of India nor their possible extension to the east to include the Rajahmundry Traps⁶⁶. The major episode of Deccan Trap emplacement has a potassium-argon age of 60–65 Myr^{65,67}. The traps of this major episode were emplaced during a single reversed magnetic polarity interval⁶⁸⁻⁷⁰. Recent palaeomagnetic and radiometric data indicate that the bulk of the traps were emplaced during the K/T reversed magnetic polarity interval 29R (refs 71, 72). Magnetic interval 29R has a duration of about 600 kyr. At the time of their emplacement palaeogeographic reconstructions place the Deccan Traps in the vicinity of the Reunion hot spot⁷³.

In addition recent DSDP studies in the Walvis Ridge region of the south-east Atlantic show a distinct peak in explosive volcanism specifically at K/T time. The Walvis Ridge is a volcanic edifice presumed to have been formed by hot spot/mantle plume activity. The K/T cores are quite spectacular and unlike cores elsewhere in the geological section at these sites. Red to green bands (high ash fraction) alternate with white to light brown bands (low ash fraction); these are underlain by white Maastrichtian chalk at sites 527 and 529 and by Maastrichtian chalk interrupted by thick green to black volcanogenic sands at site 528 (ref. 74). The K/T boundary at these sites is characterized by major mineralogical and geochemical changes indicative of intense volcanic activity over a relatively short geological time interval of 300 kyr or longer^{74,75}.

Effects of volcanism at K/T boundary

As discussed above, the geological and geochemical signatures at K/T time indicate that there was intense global volcanism over a relatively short geological interval of about 10 to 100 kyr. A number of investigators have suggested that effects associated with intense volcanism over a geologically short time period

Table 1 Summary of values used in calculations

K/T iridium flux	$63 \times 10^{-9} \text{ g Ir cm}^{-2}$
Laki flux rates	$7.17 \times 10^6 \text{ t H}_2\text{SO}_4 \text{ km}^{-3} \text{ basalt}$ $1.27 \times 10^5 \text{ t HCl km}^{-3} \text{ basalt}$
Kilauea flux rates	31 g Ir d^{-1} $10,500 \text{ t SO}_2 \text{ d}^{-1}$ 260 t HCl d^{-1} $11 \times 10^6 \text{ m}^3 \text{ basalt d}^{-1}$
Estimated K/T fluxes	$17 \times 10^{12} \text{ t H}_2\text{SO}_4$ $27 \times 10^{10} \text{ t HCl}$ $11 \times 10^6 \text{ km}^3 \text{ basalt}$
Estimated K/T flux rates	$1,700 \times 10^6 \text{ t H}_2\text{SO}_4 \text{ yr}^{-1}$ $270 \times 10^5 \text{ t HCl yr}^{-1}$ $1,100 \text{ km}^3 \text{ basalt yr}^{-1}$
Earth area	$510 \times 10^6 \text{ km}^2$
Ocean area	$360 \times 10^6 \text{ km}^2$
Ocean volume	$1,370 \times 10^6 \text{ km}^3$

were the causative agents for the K/T extinctions^{22,27,45,76-87}. In particular, Keith^{80,81} emphasized the importance of volatile emissions in the reduction of the atmospheric ozone layer and in changes in ocean chemistry, and McLean^{82,83} and Ekdale and Bromley²² suggested that the carbon dioxide emissions are important to atmospheric and oceanic changes. Also, in conjunction with estimated effects from an asteroid impact, Lewis *et al.*⁸⁸ have emphasized the potential importance of acid rain toward explaining the scope and selectivity in biological extinctions at K/T time; in their model the acid rain would have been an instantaneous event. Prinn⁸⁹, in a more recent analysis, calculated that glancing impact of a comet could produce nitrogen oxides in the atmosphere sufficient to have a significant effect on organisms.

The important consideration for evaluating the environmental effects of intense global volcanism is to have reasonable estimates for the volatile emissions particularly that of sulphur dioxide. We estimate these emissions directly from the observed iridium flux at K/T time and indirectly from the magnitude of Deccan volcanism.

Eruptions of basaltic magma release at least an order of magnitude greater SO_2 volcanic gas to the atmosphere than comparable volume eruptions of silicic magma^{90,91}. For the nonexplosive fissure eruption from Laki in 1983 Devine *et al.*⁹¹ estimate fluxes of $7.17 \times 10^6 \text{ tonnes H}_2\text{SO}_4 \text{ km}^{-3} \text{ basalt}$ and $1.27 \times 10^5 \text{ tonnes HCl km}^{-3} \text{ basalt}$. Using the magnitude of the Deccan Trap flood basalts of $1 \times 10^6 \text{ km}^3$ as an estimate of K/T volcanism total fluxes of $7.2 \times 10^{12} \text{ t H}_2\text{SO}_4$ and $13 \times 10^{10} \text{ tonnes HCl}$ are obtained.

Alvarez *et al.*⁹² have summarized the observed K/T iridium fluxes at 21 locations worldwide. The values range from 0 to $330 \times 10^{-9} \text{ g Ir cm}^{-2}$ with an average of $63 \times 10^{-9} \text{ g Ir cm}^{-2}$. The total K/T iridium flux for the entire Earth is then $63 \times 10^{-9} \times 510 \times 10^6 \times 10^{10} = 32 \times 10^{10} \text{ g Ir}$, Table 1. Olmez *et al.*⁹³ have observed flux rates for various constituents including iridium from small vent eruptions of Kilauea. Their results are 31 g Ir d^{-1} , $10,500 \text{ t SO}_2 \text{ d}^{-1}$, 260 t HCl d^{-1} and $11 \times 10^6 \text{ m}^3 \text{ basalt d}^{-1}$. Using these values and the K/T iridium value gives fluxes of $(32 \times 10^{10} \times 10,500/31) \times (98/64) = 170 \times 10^{12} \text{ t H}_2\text{SO}_4$, $270 \times 10^{10} \text{ t HCl}$ and $110 \times 10^6 \text{ km}^3 \text{ basalt}$. Olmez *et al.* note that the iridium flux shows a strong dependence on the temperature of the vent with an order of magnitude difference between the iridium released from the hotter vent as compared with the colder vent; they suggest that the iridium flux rate would be still higher for the temperatures of an erupting magma. Such higher release rates would proportionally lower the above estimates. Accordingly we estimate the K/T fluxes to be an order of magnitude less than the values calculated from the release vent data, or $17 \times 10^{12} \text{ t H}_2\text{SO}_4$, $27 \times 10^{10} \text{ t HCl}$ and $11 \times 10^6 \text{ km}^3 \text{ basalt}$. This reduction brings the H_2SO_4 and HCl fluxes into reasonable agreement with the estimates from the data of Devine *et al.*, but

there remains an order of magnitude difference in the total eruptive basalt between the two estimates.

The K/T iridium observations indicate a total temporal flux interval of 10 to 100 kyr. We do not know how the flux rate varied within this interval but the fact that it shows peaks indicates that it is reasonable to assume that there was considerable variation perhaps building to one or more peaks over shorter time intervals. Similarly from the Deccan Traps we know only that the bulk of the Traps were emplaced over a period of around 600 kyr but do not know how the eruption rate varied within this time interval. In the calculations that follow we have assumed that the fluxes were concentrated over a 10,000 yr interval rather than using a longer time estimate of 100,000 yr with superimposed peaks. This gives flux rates of $1,700 \times 10^6$ t H_2SO_4 yr^{-1} , 270×10^5 t HCl yr^{-1} and $1,100 \text{ km}^3$ basalt yr^{-1} .

As with present day fossil fuel emissions, the SO_2 injection into the atmosphere by K/T volcanism will return to the ground or enter the ocean as acid rain. The 1976 fossil fuel emissions of SO_2 were 28×10^6 t yr^{-1} for the United States and 53×10^6 t yr^{-1} for Europe, giving an equivalent H_2SO_4 rain of $(28 + 53) \times 10^6 \times (98/64) = 120 \times 10^6$ t yr^{-1} (ref. 94). The K/T volcanic acid rain would be $1,700/120 = 14$ times greater. This is a global average value. The acid rain for any given location or time could be substantially in excess of this value. If we project from the recognized effects of present day acid rain, it seems apparent that the greater concentrations at K/T time would have a significant effect on terrestrial fauna and flora.

One of the more important biochemical properties of the ocean is its buffering capacity. It takes a large amount of hydrogen ion input to significantly alter ocean alkalinity. Ocean alkalinity is defined as $A = \text{HCO}_3^- + 2\text{CO}_3^{2-}$. For the upper ocean above the main thermocline at around 100 m, the bicarbonate ion concentration is around 1.8 g-at m^{-3} and the carbonate ion concentration around 0.25 g-at m^{-3} for an alkalinity of 2.3 g-at m^{-3} (ref. 95). For the deep ocean the alkalinity is around 2.5 g-at m^{-3} . The titration effect of the H_2SO_4 hydrogen ion flux from K/T volcanism will be first to reduce all the available carbonate ion to bicarbonate ion and then to reduce the bicarbonate ion to carbonic acid⁹⁶. For a reduction of all the carbonate ion in the upper ocean layer, the equilibrium between the surface ocean and the atmosphere would lead to a corresponding reduction in pH from a normal value of 8.2 to around 7.4 or possibly less⁹⁷.

The total hydrogen ion flux to the ocean from K/T volcanism will be $17 \times 10^{12} \times 10^6 \times (2/98) = 35 \times 10^{16}$ g-at. This flux will lead to a gradual reduction in alkalinity for the whole ocean by up to an amount of $35 \times 10^{16}/1370 \times 10^6 \times 10^9 = 0.26 \text{ g-at m}^{-3}$ over the 10,000 yr period. An alternative approach is to consider flux rates into and out of the upper ocean above the main thermocline with the deep ocean as an infinite sink. The hydrogen ion flux rate will be $1,700 \times 10^6 \times 10^6 \times (2/98)/360 \times 10^6 \times 10^6 = 0.10 \text{ g-at m}^{-2} \text{ yr}^{-1}$. For an upper ocean thickness of 100 m and a residence time of 50 yr⁹⁵, the reduction in alkalinity would be $0.10 \times 50/100 = 0.05 \text{ g-at m}^{-3}$. The first calculation does not include time-dependent feedback effects which would reduce the estimate. The second calculation does not include hydrogen ion exchanges from the deep ocean which would increase the estimate, and palaeoceanography suggests that vertical circulation of the ocean was more sluggish at K/T time⁹⁸ which would also increase the estimate. Within these constraints it does appear that the K/T hydrogen ion flux to the ocean would be sufficient to product a noticeable change in pH particularly for the surface ocean waters.

Global cooling of Earth surface temperature is a known effect from large volcanic eruptions^{90,99-102}. The observed cooling is related to the volcanic aerosols, principally SO_2 , which have a stratospheric residence time of 1-2 yr as compared with the silicate dust which falls out within a few months^{90,103-108}. While most of the observations have been of explosive volcanoes, Stothers *et al.*¹⁰⁹ and Rampino *et al.*¹¹⁰ have calculated that large volume fissure eruptions are capable of injecting large quantities

of sulphate aerosols into the lower stratosphere. From the time dependent relation for aerosols in the atmosphere given by Hansen *et al.*¹⁰³, the average residence time is 1.2 yr. The amount of SO_2 in the atmosphere from K/T volcanism would be $1,700 \times 10^6 \times 1.2 \times (64/98) = 1,300 \times 10^6$ t. Extrapolating from the empirical relation between observed surface cooling and sulphur emissions from large historic eruptions given by Devine *et al.*⁹¹, a global cooling of 3.1°C would be expected. It is important to emphasize that this cooling estimate is an extrapolation from the effects of known volcanic eruptions and that it presumes the validity of the calculations for large volume basaltic fissure eruptions of Stothers *et al.*¹⁰⁹ and Rampino *et al.*¹¹⁰. One of the anticipated effects of this cooling would be that the replacement species following an extinction would be of a higher latitude and harder form than their predecessors.

HCl aerosols injected into the stratosphere from historic volcanic eruptions have led to a decrease in the protective ozone layer¹¹¹⁻¹¹³. In discussing the effects of the Krakatoa and Agung eruptions Stolarski and Butler¹¹³ predict a global ozone layer depletion of 7.7% for a stratospheric injection of 300×10^6 kg HCl. For the HCl produced from K/T volcanism the average atmospheric content would be $270 \times 10^5 \times 10^3 \times 1.2 = 32,000 \times 10^6$ kg HCl. This is $32,000/300 = 110$ times greater than that for a 7.7% reduction in the ozone layer and would suggest a serious depletion of the ozone layer at K/T time. The principal biological effect of the resultant increase in ultraviolet radiation would be on those terrestrial animals that were unprotected or unable to hide in either their adult or reproductive stages.

Taking a value of 300 p.p.m. for carbon released as CO_2 from volcanic eruptions¹¹⁴ and a density of 2.8 g cm^{-3} for basalt, the K/T carbon flux to the atmosphere will be $300 \times 10^{-6} \times 2.8 \times 1,100 \times 10^{15} = 0.9 \times 10^{15} \text{ g yr}^{-1}$. The present anthropogenic flux of fossil fuel carbon emissions to the atmosphere is $5 \times 10^{15} \text{ g yr}^{-1}$ and that from terrestrial biota $53 \times 10^{15} \text{ g yr}^{-1}$ (ref. 115). McLean⁸³ argues that the cumulative effect of volcanic CO_2 over 500,000 yr can be substantial although feedback mechanisms over that time period could seriously affect this model. In any event the effect of increased CO_2 would also be to reduce ocean alkalinity.

The sulphate ion input to the ocean from K/T volcanism will be $17 \times 10^{12} \times (96/98) = 17 \times 10^{12}$ t. The sulphate ion concentration in the ocean is 28 g-at m^{-3} (ref. 95), giving a total sulphate content for the whole ocean of 3.7×10^{15} t. The K/T volcanic sulphate flux to the ocean is small compared with the ocean reservoir.

An additional consideration is what global geological record such a period of increased volcanism might leave. Here we wish to consider the global record exterior to the immediate regions of basalt or volcanic ash emplacement. Deirmendjian¹¹⁶ estimated that the explosive volcanic eruption of Krakatoa injected an amount of fine dust into the atmosphere of 0.17% of the total eruption volume. For K/T volcanism we have presumed that the bulk of the volcanism was of a basaltic type. If we assume that 1/4 of the K/T volcanism was explosive a global fine dust layer of $11 \times 10^6 \times 0.0017 \times 10^5/4 \times 510 \times 10^6 = 0.9 \text{ cm}$ would result. The global record of K/T volcanism would be small although local effects could be significant.

An extinction model

We have suggested that a substantial increase in volcanism at K/T time would produce acid rain, decrease in surface ocean alkalinity, global temperature change and ozone layer depletion. In addition there is associated with the late Cretaceous a pronounced regression of the sea lasting for a period of a few million years^{117,118}. Evidence for this regression is primarily stratigraphic¹¹⁷⁻¹¹⁹ but it is also supported by geochemical data^{120,121}. The seawater ratio of $^{87}\text{Sr}/^{86}\text{Sr}$ shows a gradual increase in the late Cretaceous continuing into the Tertiary, interrupted by a sharp rise and fall at the end of the Cretaceous. Rise in the ratio relates to increase in continental runoff and is

most plausibly explained in terms of increased continental area due to sea level fall^{117,122}. Regression of the sea during the late Cretaceous would also affect climate. Pitman¹²³ explains the sea level regression in terms of ocean spreading ridge volume changes which are related to changes in the rate of seafloor spreading. Other possibilities related to plate tectonic activity include change in the length of the ocean ridge system and subsidence of intraplate volcanic mountains in the Pacific¹¹⁷. We consider that the major regression of the sea during the late Cretaceous, explained in terms of plate tectonics, and the K/T volcanism, explained in terms of hot spot/mantle plume activity, must be generically related.

The crux for any scenario of late Cretaceous and K/T environmental events is how well it explains the faunal and floral changes that we see in the geologic record. It is not only important that the model provide an explanation for those species that became extinct; it must also account for those which showed little or no change across the K/T transition. We list below the observed phenomena together with suggested causes in terms of the above factors.

The carbonate dependent planktonic organisms, foraminifera and coccolithophoridae, of the surficial ocean waters show major extinctions at K/T time^{20,124,125}. While both groups declined abruptly at the end of the Cretaceous, the main coccolith extinctions occurred a few thousand years later^{4,21}. Modern planktonic foraminifera become inhibited below a pH of 7.6–7.8 (refs 83, 126, 127), and modern coccolithophoridae do not survive below a pH of 7.0–7.3 (refs 83, 128–130). If K/T planktonic forms were similarly affected, the changes in pH resulting from volcanism at K/T time would lead to extinction effects for both foraminifera and coccolithophoridae with the foraminiferal extinctions preceding those of the coccoliths.

The earliest Tertiary carbonate dependent plankton show a change from large and ornate forms to small and simple forms^{4,131}. For modern plankton small forms survive better in a lower pH environment than large forms¹²⁷, and an environment which is undergoing change is selective toward smaller species as compared with a more stable environment¹³². The anticipated pH and environmental conditions for the K/T surface ocean waters would be preferential to the smaller forms.

The earliest Tertiary calcareous micro and nannofossil replacement forms are of a higher latitude, colder water type than their predecessors⁴. Higher latitude forms are generally more robust than their warm water counterparts. We would expect that the major effects of decreased pH would be in the tropical and temperate surface waters and that any effects of global cooling would favour survival of the higher latitude and harder forms.

Dinoflagellates are the least affected of the plankton across the K/T transition^{125,133,134}. Diatoms, silicoflagellates and radiolarians are not as numerous in Upper Cretaceous and Lower Tertiary sediments as the dinoflagellates, and tabulations differ as to the changes in these planktonic forms^{125,134}. Dinoflagellates are not dependent on carbonate. In freshwater acid lakes with a pH of 4.0–5.0 dinoflagellates are one of the few surviving planktonic groups¹³⁵. We would expect that the pH conditions of the K/T surface ocean waters would have less effect on the dinoflagellates than on the carbonate dependent plankton.

Deep water benthic foraminifera in the oceans show little change across the K/T transition^{124,125,133}. It would be expected that alkalinity and pH changes would be greatest in the surface waters especially if the Cretaceous ocean were more strongly stratified than the modern ocean as would appear to have been the case⁹⁸. We would expect that the K/T environmental changes would be smaller for the deep ocean with less effect on the benthic foraminifera.

There is a peak in calcite dissolution for shallow water forms at K/T time^{22,136}, and some DSDP sites show evidence of dissolution of some of the micro and nannofossils⁴. The former would be expected for the anticipated changes in alkalinity of

the upper ocean and shallow waters; the latter may be related to changes in the calcite compensation depth associated with the anticipated smaller decrease in deep ocean alkalinity.

For shallow water macrofauna the most striking changes took place a few million years before the end of the Cretaceous^{7–9}. Shallow water macrofauna and especially reef communities would be expected to be affected by sea level changes. While there is evidence of extinction at the boundary (and the problem of lack of complete sections across the boundary in shallow water facies has already been alluded to), it could relate to a collapse of the food web and requires no special explanation. Eurytopic shallow water benthos that had survived the earlier perturbations of the physical environment that led to the extinction of the rudists and other stenotopic groups would be expected to be relatively resistant to extinction. The key issue for the marine realm therefore concerns the plankton.

There is dispute about whether the dinosaurs had already been in decline prior to when they finally became extinct^{14,18,131,137–140}. What should not be in dispute is that, as generally large organisms with small populations, dinosaur species were probably always relatively vulnerable to extinction; and the importance of the terminal Cretaceous period lay primarily in the failure of new dinosaur groups to evolve and occupy the niches vacated by the extinct forms, as had happened many times previously in the Mesozoic. We speculate, as have several other investigators^{18,137,138,140}, that sea level regression led to an increase in continental type climates with greater seasonal extremes of temperature. This environmental stress may possibly have been reinforced by the increase in ultraviolet radiation at K/T time.

Placental mammals, birds, and freshwater amphibians and reptiles survived the K/T transition with little to no change^{137–141} which implies that changes in the continental environment were somewhat less drastic for many groups. It is difficult to perceive a common protective element for such diverse organisms. Different factors may be speculated upon for different cases, for example, large population size, wide temperature tolerance, and escape from ultraviolet radiation by nocturnal habits and subaquatic or subterranean habitats.

Freshwater fish showed selective extinctions¹³⁹. These changes may be related to the effects of acid rain. Modern freshwater fish in a Canadian lake which has undergone severe changes in pH from acid rain show selective extinctions with, for example, walleye and lake trout going out at a pH of 5.8–5.2, northern pike and white sucker going out at a pH of 5.2–4.7, and yellow perch and lake herring surviving at a pH of less than 4.7 (ref. 142). If these examples apply in principle to K/T freshwater fish, extinctions of a selective nature would be expected.

Terrestrial floral changes were also selective and occurred over an extended time interval^{11,15,16,140,143}. At the iridium anomaly horizon in North America, however, there is a sharp palynological change indicative of a decline of angiosperms at the expense of ferns¹⁴⁴. Hickey¹⁵ suggests that climatic changes during the late Cretaceous may be the important longer term environmental factor but it is also possible that acid rain at K/T time may have been a contributive factor to account for the shorter term floral changes^{16,144}. Modern flora in Canada from regions subject to acid rain show selective effects with, for example, white pine and white birch being sensitive, red pine and balsam fir showing intermediate effects, and red cedar and sugar maple being tolerant¹⁴⁵. If similar species tolerance existed at K/T time, extinctions of a selective nature would be expected.

Conclusions

The selective nature of the extinction record during the late Cretaceous and at K/T time does not support a model which calls upon a decrease in solar radiation due to dust in the atmosphere or stratosphere, and no other comprehensive scenario solely related to impact has been proposed. We have

proposed a scenario that calls upon a substantial increase in volcanic activity over at least 10,000 yr at K/T time. The volatile emissions from this volcanism would lead to acid rain, reduction in the alkalinity and pH of the surface ocean, global atmospheric cooling, and ozone layer depletion. These effects coupled with those associated with the major sea level regression of the late Cretaceous provide the framework for a reasonable explanation of the extinction record.

The mass extinction of calcareous plankton, which is a more striking and enigmatic phenomenon than the more widely publicized extinction of the dinosaurs, together with correlative extinctions in other groups may be viewed as an environmental *coup de grace* following a longer period of increased environmental stress associated with reduced habitat area and a deteriorating temperature regime.

Note added in proof: We note two recent studies related to K/T events. H. J. Hansen *et al.* *Bull. geol. Soc. Denmark* **35**, 75-82 (1986), examined microspherules from Denmark, New Zealand and Spain. The microspherules are of a goethite or K-feldspar and goethite composition. After dissolution in HCl and HF, organic spheres were found which according to their morphology and structure appear to belong to the green algal group *Prasinophyta*. The authors conclude that the microspherules are the result of diagenetic infill of prasinophyte algae. D. E. Loper and K. McCartney *Geophys. Res. Lett.* **13**, 1525-1528 (1986) and P. Olson *et al.* *Nature* (in the press), conjecture that episodes of volcanism including that at K/T time are related to instabilities in the thermal boundary layer at the base of the mantle and that these instabilities produce increased mantle plume activity on a global scale.

1. Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. *Science* **208**, 1095-1108 (1980).
2. Alvarez, W. *et al.* *Science* **223**, 1135-1141 (1984).
3. Kennett, J. P. *Marine Geology* (Prentice Hall, New York, 1982).
4. Perch-Nielsen, K., McKenzie, J. & He, Q. *Spec. Pap. geol. Soc. Am.* **190**, 353-371 (1982).
5. Smit, J. *Spec. Pap. geol. Soc. Am.* **190**, 329-352 (1982).
6. Ward, P. D. & Signor, P. W. in *Phanerozoic Diversity Patterns* (ed. Valentine, J. W.) 399-418 (Princeton University Press, 1985).
7. Mount, J. F., Margolis, S. V., Showers, W., Ward, P. & Doehne, E. *Palaios* **1**, 87-92 (1986).
8. Ward, P., Wiedmann, J. & Mount, J. F. *Geology* **14**, 899-903 (1986).
9. Kauffman, E. G. in *Catastrophes and Earth History* (eds Berggren, W. A. & Van Couvering, J. A.) 151-246 (Princeton University Press, 1984).
10. Lerbeckmo, J. F., Evans, M. E. & Baadsgaard, H. *Nature* **279**, 26-30 (1979).
11. Hickey, L. J. *Nature* **292**, 529-531 (1981).
12. Archibald, J. D. *Nature* **291**, 650-652 (1981).
13. Archibald, J. D., Butler, R. F., Lindsay, E. H., Clemens, W. A. & Dingus, L. *Geology* **10**, 153-159 (1982).
14. Sloan, R. E. & Van Valen, L. *Science* **148**, 220-227 (1965).
15. Hickey, L. J. in *Catastrophes and Earth History* (eds Berggren, W. A. & Van Couvering, J. A.) 279-313 (Princeton University Press, 1984).
16. Tshudy, R. H. & Tshudy, B. D. *Geology* **14**, 667-670 (1986).
17. Padian, K. & Clemens, W. A. in *Phanerozoic Diversity Patterns* (ed. Valentine, J. W.) 41-96 (Princeton University Press, 1985).
18. Sloan, R. E., Rigby, J. K., Jr, Van Valen, L. M. & Gabriel, D. *Science* **232**, 629-633 (1986).
19. Officer, C. B. & Drake, C. L. *Science* **219**, 1383-1390 (1983).
20. Thierstein, H. R. *Spec. Publ. Soc. econ. Paleont. Miner.* **32**, 355-394 (1981).
21. Smit, J. & Romein, A. J. T. *Earth planet. Sci. Lett.* **74**, 155-170 (1985).
22. Ekdale, A. A. & Bromley, R. G. *J. sedim. Petrol.* **54**, 681-703 (1985).
23. Arthur, M. A. & Fischer, A. G. *Bull. geol. Soc. Am.* **88**, 367-371 (1977).
24. Rampino, M. R. & Reynolds, R. C. *Science* **219**, 495-498 (1983).
25. Johnsson, M. & Reynolds, R. C. *J. sedim. Petrol.* **56**, 501-509 (1986).
26. Jeffers, J. D. thesis, Dartmouth College, Hanover, New Hampshire (1985).
27. Officer, C. B. & Drake, C. L. *Science* **227**, 1161-1167 (1985).
28. Crockett, J. D., Officer, C. B., Johnson, G. D. & Wezel, F. C. *Geology* (submitted).
29. Rocchia, R., Renard, M., Bolet, D. & Bonté, Ph. *Bull. Soc. géol. Fr.* **26**, 1193-1202 (1984).
30. Lerbeckmo, J. F. & St. Louis, R. M. *Can. J. Earth Sci.* **23**, 120-124 (1986).
31. Michel, H. V., Asaro, F., Alvarez, W. & Alvarez, L. W. *Init. Rep. DSDP* **62**, 847-849 (1981).
32. Hsu, K. J. *et al.* *Science* **216**, 249-256 (1982).
33. Michel, H. V., Asaro, F., Alvarez, W., Alvarez, L. W. & Johnson, D. A. *Init. Rep. DSDP* **72**, 931-936 (1984).
34. Michel, H. V., Asaro, F., Alvarez, W. & Alvarez, L. W. *Init. Rep. DSDP* **86**, 533-538 (1985).
35. Reinhardt, J., Schindler, J. S. & Donovan, A. D. *Geol. Soc. Am. Ann. Meet. Abs.*, 728 (1986).
36. Hansen, T. A. & Kaufman, E. G. *Geol. Soc. Am. Ann. Meet. Abs.*, 627 (1986).
37. Maurrasse, J.-M. R. *Geol. Soc. Am. Ann. Meet. Abs.*, 686 (1986).
38. Zoller, W. H., Parrington, J. R. & Phelan Kotra, J. M. *Science* **222**, 1118-1121 (1983).
39. Smit, J. & Klaver, G. *Nature* **292**, 47-49 (1981).
40. De Paolo, D. J., Kytte, F. T., Marshall, B. D., O'Neill, J. R. & Smit, J. *Earth planet. Sci. Lett.* **64**, 356-373 (1983).
41. Varekamp, J. C. & Thomas, E. *Spec. Pap. geol. Soc. Am.* **190**, 461-467 (1982).
42. Montanari, A. *et al.* *Geology* **11**, 668-671 (1983).
43. Naslund, H. R., Officer, C. B. & Johnson, G. D. *Geology* **14**, 923-926 (1986).
44. Brooks, R. R., Hoek, P. L., Reeves, R. D., Wallace, R. C., Johnston, J. H., Ryan, D. E., Holzbecher, J. & Collen, J. D. *Geology* **13**, 738-740 (1985).
45. Wezel, F. C., Vannucci, S. & Vannucci, R. *C. r. heb. Séanc. Acad. Sci. Paris* **293**, 837-844 (1981).
46. Vannucci, R., Vannucci, S., Mazzucotelli, A., Meloni, S. & Oddone, M. *Rend. Soc. Ital. Mineral. Petrol.* **38**, 413-422 (1981).
47. Montanari, A. *Geology* **14**, 1024-1026 (1986).
48. Fredriksson, K. & Martin, L. R. *Geochim. cosmochim. Acta* **27**, 245-248 (1963).
49. Wright, F. W. & Hodge, P. W. *J. geophys. Res.* **70**, 3889-3898 (1965).
50. Bohor, B. F., Foord, E. E., Modreski, P. J. & Triplehorn, D. M. *Science* **224**, 867-869 (1984).
51. Izett, G. A. & Pillmore, C. L. *Geol. Soc. Am. Ann. Meeting Abs.*, 617 (1985).
52. Nichols, D. J., Jarzen, D. M., Orth, C. J. & Oliver, P. Q. *Science* **231**, 714-717 (1986).
53. Bohor, B. F., Modreski, P. J. & Foord, E. E. *Abstracts of papers Sixteenth Lunar and Planetary Science Conference*, 79-80 (1985).
54. Preisinger, A. *et al.* *Nature* **322**, 794-799 (1986).
55. French, B. M. & Short, N. M. (eds) *Shock Metamorphism of Natural Materials* (Mono Books, Baltimore, 1968).
56. Izett, G. A. & Pillmore, C. L. *EOS* **66**, 1149-1150 (1985).
57. Lilly, P. A. *J. geophys. Res.* **86**, 10689-10700 (1981).
58. Simpson, C. *J. geophys. Res.* **86**, 10701-10706 (1981).
59. Schreyer, W. J. *Petrol.* **24**, 26-47 (1983).
60. Carter, N. L., Officer, C. B., Chesner, C. A. & Rose, W. I. *Geology* **14**, 380-383 (1986).
61. Carter, N. L. & Officer, C. B. *Geology* **15**, 91-92 (1987).
62. Carter, N. L. & Drake, C. L. *Earth planet. Sci. Lett.* (submitted).
63. Kent, D. V. *Science* **211**, 648-650 (1981).
64. Izett, G. A., Wilcox, R. E., Powers, H. A. & Desborough, G. A. *Quat. Res.* **1**, 121-132 (1970).
65. Kaneoka, I. & Haramura, H. *Earth planet. Sci. Lett.* **18**, 229-236 (1973).
66. Baksi, A. J. *Eos* **66**, 1145 (1985).
67. Wellman, P. & McElhinney, M. W. *Nature* **227**, 595-596 (1970).
68. Wensink, H. & Klootwijk, C. T. *Tectonophysics* **11**, 175-190 (1971).
69. Klootwijk, C. T. in *Geodynamics of Pakistan* (eds Farah, A. & De Jong, K. A.) 41-80 (Geological Survey of Pakistan, Quetta, 1979).
70. Wensink, H. *et al.* *Fourth International Gondwana Symposium* (eds Laskar, B. & Rajarao, C. S.) 832-849 (Hindustani Publishing, Delhi, 1979).
71. Courtillot, V., Besse, J., Vandamme, D., Jaeger, J.-J. & Montigny, R. *C. r. heb. Séanc. Acad. Sci. Paris* **303**, 863-867 (1986).
72. Courtillot, V., Besse, J., Vandamme, D., Montigny, R., Jaeger, J.-J. & Capetta, H. *Earth planet. Sci. Lett.* **80**, 361-374 (1986).
73. Morgan, W. J. *Bull. Am. Ass. Petrol. Geol.* **56**, 203-213 (1972).
74. Borella, P. E. *Init. Rep. DSDP* **74**, 645-662 (1984).
75. Chamley, H., Maillot, H., Duée, G. & Robert, C. *Init. Rep. DSDP* **74**, 685-695 (1984).
76. Axelrod, D. I. *Spec. Pap. geol. Soc. Am.* **185**, 1-59 (1981).
77. Campsie, J., Johnson, G. L., Jones, J. E. & Rich, J. E. *Eos* **65**, 796-800 (1984).
78. Doreen, J. M. *Microfal.* **20**, 178-193 (1974).
79. Gledhill, J. A. *Eos* **66**, 153 (1985).
80. Keith, M. L. *Eos* **61**, 400 (1980).
81. Keith, M. L. *Geochim. cosmochim. Acta* **46**, 2621-2637 (1982).
82. McLean, D. M. *Eos* **63**, 562 (1982).
83. McLean, D. M. *Am. Geophys. Un. Geophys. Mon.* **32**, 493-503 (1985).
84. Möerner, N.-A. *J. Geol.* **90**, 564-573 (1982).
85. Van Valen, L. M. *Paleobiology* **10**, 121-137 (1984).
86. Vogt, P. R. *Nature* **240**, 338-342 (1972).
87. Rich, J. E., Johnson, G. L., Jones, J. E. & Campsie, J. *Paleocean.* **1**, 85-95 (1986).
88. Lewis, J. S., Watkins, G. H., Hartman, H. H. & Prinn, R. G. *Spec. Pap. geol. Soc. Am.* **190**, 215-221 (1982).
89. Prinn, R. G. *Eos* **66**, 813 (1985).
90. Sigurdsson, H. *Eos* **63**, 601-602 (1982).
91. Devene, J. D., Sigurdsson, H., Davis, A. N. & Self, S. *J. geophys. Res.* **89**, 6309-6325 (1984).
92. Alvarez, W., Alvarez, L. W., Asaro, F. & Michel, H. V. *Spec. Pap. geol. Soc. Am.* **190**, 305-315 (1982).
93. Olmze, I., Finnegan, D. L. & Zoller, W. H. *J. geophys. Res.* **91**, 653-663 (1986).
94. Likens, G. E., Wright, R. F., Galloway, J. N. & Butler, T. J. *Scient. Am.* **241**, 34-51 (1979).
95. Broecker, W. S. *Chemical Oceanography* (Harcourt, New York, 1974).
96. MacIntyre, F. *Sci. Am.* **223**, 104-115 (1970).
97. Sverdrup, H. V., Johnson, M. W. & Fleming, R. H. *The Oceans* (Prentice Hall, New York, 1942).
98. Berger, W. H. *Am. Geophys. Un. Maurice Ewing Ser.* **3**, 297-314 (1979).
99. Self, S., Rampino, M. R., Newton, M. W. & Wolff, J. A. *Geology* **12**, 659-663 (1984).
100. Simkin, T. & Fiske, R. S. *Eos* **67**, 513-514 (1983).
101. Stommel, H. & Stommel, E. *Scient. Am.* **240**, 176-186 (1979).
102. Wood, C. A. *Eos* **65**, 410 (1984).
103. Hansen, J. E., Wang, W.-C. & Lacis, A. A. *Science* **199**, 1065-1068 (1978).
104. Kelly, P. M. & Sear, C. B. *Nature* **311**, 740-743 (1984).
105. Pollack, J. B., Toon, O. B., Sagan, C., Summers, A., Baldwin, B. & Van Camp, W. J. *geophys. Res. Lett.* **81**, 1071-1083 (1976).
106. Rampino, M. R. & Self, S. *Quat. Res.* **18**, 127-143 (1982).
107. Rampino, M. R. & Self, S. *Nature* **310**, 677-679 (1984).
108. Toon, O. B. & Pollack, J. B. *Am. Sci.* **68**, 268-278 (1980).
109. Stothers, R. B., Wolff, J. A., Self, S. & Rampino, M. R. *Geophys. Res. Lett.* **13**, 725-728 (1986).
110. Rampino, M. R., Stothers, R. B., Wolff, J. A. & Self, S. *Geol. Soc. Am. Ann. Meet. Abs.*, 725 (1986).
111. Johnston, D. A. *Science* **209**, 491-493 (1980).
112. Stolarski, R. S. & Cicerone, R. J. *Can. J. Chem.* **52**, 1610-1615 (1974).
113. Stolarski, R. S. & Butler, D. M. *Pure Appl. Geophys.* **117**, 486-497 (1979).
114. Anderson, A. T. *Rev. Geophys. Space Phys.* **13**, 37-55 (1975).
115. Revelle, R. & Munk, W. in *Energy and Climate* (ed. Revelle, R.) 140-158 (Nat. Acad. Sci., Washington, 1977).
116. Deirmendjian, D. *Adv. Geophys.* **16**, 267-296 (1973).
117. Hallam, A. *Rev. Earth Planet. Sci.* **12**, 205-243 (1984).
118. Newell, N. D. *Spec. Pap. geol. Soc. Am.* **190**, 257-263 (1982).
119. Haq, B. U., Hardenpol, J. & Vail, P. R. *Science* (in the press).
120. Koepnick, R. B., Burke, W. H., Denison, R. E., Hetherington, E. A., Nelson, H. F., Otto, J. B. & Waite, L. E. *Chem. Geol.* **58**, 55-81 (1985).
121. Hess, J., Bender, M. L. & Schilling, J.-G. *Science* **231**, 979-984 (1986).
122. Hallam, A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **47**, 195-223 (1984).
123. Pitman, W. C. *Bull. geol. Soc. Am.* **89**, 1389-1403 (1978).
124. Thierstein, H. R. in *Climate in Earth History* (eds Berger, W. H. & Crowell, J. C.) 90-95 (Nat. Acad. Sci., Washington, 1982).
125. Thierstein, H. R. *Spec. Pap. geol. Soc. Am.* **190**, 385-399 (1982).
126. Phleger, F. B. & Bradshaw, J. S. *Science* **154**, 1551-1553 (1966).
127. Boltovskoy, E. & Wright, R. *Recent Foraminifera* (Junk, The Hague, 1976).
128. Sikes, C. S. & Wilbur, K. M. *J. Phycol.* **16**, 433-436 (1980).
129. Sikes, C. S., Roer, R. D. & Wilbur, K. M. *Limnol. Oceanogr.* **25**, 248-261 (1980).

130. Sikes, C. S. & Wilbur, K. M. *Limnol. Oceanogr.* **27**, 18–26 (1982).
131. Russell, D. A. *Scient. Am.* **246**, 58–65 (1982).
132. Kilham, P. & Kilham, S. S. in *The Physiological Ecology of Phytoplankton* (ed. Morris, I.) 571–597 (Univ. California Press, Berkeley, 1980).
133. Tappan, H. *Spec. Pap. geol. Soc. Am.* **190**, 265–275 (1982).
134. Russell, D. A. *Cretaceous-Tertiary Extinctions and Possible Terrestrial and Extraterrestrial Causes* (eds Russell, D. A. & Rice, G.) 41–42 (Nat. Museums Can., Ottawa, 1982).
135. Gensemer, R. W. & Kilham, S. S. *Can. J. Fish. aquat. Sci.* **41**, 1240–1243 (1984).
136. Worsley, T. *Spec. Publ. Soc. econ. Paleont. Miner.* **20**, 94–125 (1974).
137. Archibald, J. D. & Clemens, W. A. *Am. Sci.* **70**, 377–385 (1982).
138. Clemens, W. & Archibald, J. D. *Mem. Soc. Geol. France* **139**, 67–74 (1980).
139. Russell, D. A. *Rev. Earth planet. Sci.* **7**, 163–182 (1979).
140. Van Valen, L. & Sloan, R. E. *Evol. Theory* **2**, 37–64 (1977).
141. Hutchison, J. H. & Archibald, J. D. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (in the press).
142. Beamish, R. J., Lockhart, W. L., VanLoon, J. C. & Harvey, H. H. *Ambio* **4**, 98–102 (1975).
143. Hickey, L. J., West, R. M., Dawson, M. R. & Choi, D. K. *Science* **221**, 1153–1156 (1983).
144. Orth, C. J., Gilmore, J. S., Knight, J. D., Pillmore, C. L., Tschudy, R. H. & Fassett, J. E. *Spec. Pap. geol. Soc. Am.* **190**, 423–433 (1982).
145. Linzon, S. N. *Sulfur in the Environment, Part II, Ecological Impacts* (ed. Nriagu, J. O.) 109–162 (Elsevier, Amsterdam, 1978).

ARTICLES

Molecular cloning of a protective antigen of schistosomes

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The complementary DNA sequence encoding the M_r 28,000 antigen of Schistosoma mansoni has been isolated and expressed in Escherichia coli. Experimental vaccination of rats, hamsters and monkeys with a recombinant fusion protein induces a strongly cytotoxic antibody response. Immunization of rats and hamsters with this protein leads to significant protection against a natural challenge infection with live cercariae.

SCHISTOSOMIASIS is a chronic debilitating disease affecting human beings throughout 70 countries in several parts of the world. It is estimated that 200–300 million people are infected by various species of schistosomes. In spite of the development of active and relatively safe drugs, prevention of rapid reinfection has remained a problem, requiring repeated drug applications over a long period. There is a clear need therefore for alternative control measures and in particular for an effective vaccine inducing significant levels of protection against the invasive stage of the parasite.

Following the identification of potential mechanisms of immunity in man and experimental animal models, several schistosomal antigens have been characterized and their protective activity assessed either by passive transfer of the corresponding antibodies or by direct immunization with purified antigenic preparations. Passive transfer of immune sera^{1,2} and experimental vaccination using either immunoaffinity purified antigens³ or gel filtration purified proteins^{4,5} have been shown to induce statistically significant levels of protection.

We have described a *Schistosoma mansoni* antigen with an apparent relative molecular mass of 28,000 (M_r 28K) which could be detected on the schistosomulum surface and was among the *in vitro* translation products from larval and adult stage RNA⁶. Several groups^{7–9} have identified cloned molecules of 27–28K but there is no evidence of identity with the molecule described in this paper. A significant level of protection was achieved by immunization of Fischer rats and BALB/c mice with a gel purified protein fraction of M_r 28K (ref. 10).

Here we report the cloning and expression in *E. coli* of the complementary DNA encoding a 28K antigen present in extracts of adult worms. Immunization of rats, hamsters and monkeys with a recombinant protein containing the major epitopes of the native 28K antigen induces high levels of serum cytotoxicity towards schistosomula. In addition, rats and hamsters have been shown to be protected against a challenge infection to a degree comparable to that observed after immunization with the native molecule. The 28K antigen was also shown to be present in extracts of related species like *Schistosoma bovis*, *S. haematobium*

and *S. japonicum*, demonstrating that an effective vaccine against both bovine and human schistosomiasis could be elaborated.

Cloning the cDNA encoding the 28K antigen

Total RNA from adult worms was used to synthesize double-stranded cDNA. Following nuclease S1 treatment, the cDNA was fractionated on linear sucrose gradients, and the size fractions between 500 and 4,000 base pairs (bp) were pooled. Extremities were extended with 10–15 dG residues and DNA was inserted into λ gt10¹¹ using the synthetic adapter 5'-AATTCcccccccccc-3'¹². Phages containing inserts were selected and amplified on *E. coli* POP101¹³, resulting in a library of 2×10^6 independent recombinants. Phage DNA was prepared after two CsCl equilibrium centrifugations and inserts obtained after *Eco*RI digestion were recovered from sucrose gradients by pooling the size fraction between 500 and 2,000 bp. DNA was ligated into the *Eco*RI site of the β -galactosidase gene present in λ gt11, packaged *in vitro* and samples of the library containing a total of 1×10^8 independent recombinants were plated directly on *E. coli* Y1090.

Screening was done at high density using both rat and rabbit polyclonal monospecific antibodies against the 28K antigen. Replicates were developed by subsequent incubation with biotinylated anti-rat or anti-rabbit antibodies and a biotin-peroxidase complex. Final staining in the presence of a colour reagent resulted in clearly detectable signals. From a first screening, three independent candidates λ TG06, λ TG08 and λ TG09 were selected for further characterization by sequencing the cDNA inserts in M13 derivatives using the chain-termination reaction. An oligonucleotide was synthesized based on the sequence of the cDNA insert of λ TG06 and used to screen the λ gt10 library made with RNA from adult worms. Two additional candidates, λ TG10 and λ TG11, were isolated and sequenced as above. All five cDNA inserts contained overlapping sequences (Fig. 1). Assembling individual fragments gave a longest possible cDNA of 783 bases followed by a poly(A) track. Translation of the sequence starting with a Met residue at nucleotide position 7, gave a predicted protein of 211 amino acids with M_r 28,000.

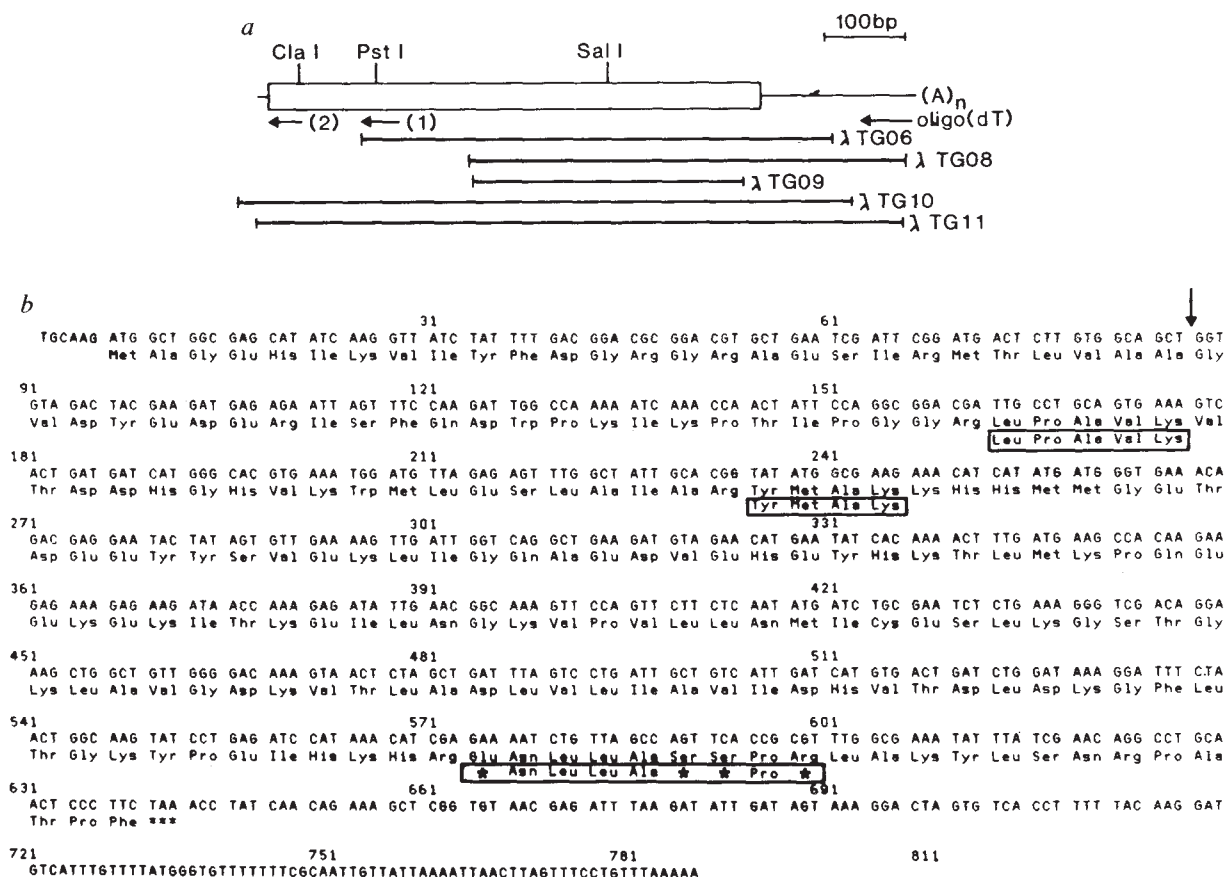


Fig. 1 *a*, Positions of the independent cDNAs relative to the presumptive messenger coding for a 28K protective antigen of *S. mansoni*. Arrows (1) and (2), synthetic oligonucleotides. *b*, Complete structure of the assembled cDNA encoding a 28K protective immunogen present in total soluble extracts of adult worms. The predicted protein sequence is shown below the DNA. Arrow, cleavage site for a putative signal peptide. The sequence of three peptides, obtained after a tryptic digest on the native protein (see Fig. 2), could be matched with the amino-acid sequence deduced from the cDNA (boxes; *, unidentified residue).

Methods. Recombinant phages λTG06, λTG08 and λTG09 originate from an adult worm λgt11 library which was screened with a polyclonal rat or rabbit antibody raised against the native antigen eluted from SDS-polyacrylamide gels. The two cDNAs, λTG10 and λTG11, containing a complete copy of the coding region, were isolated after rescreeing a λgt10 library with the synthetic oligomer (1) complementary to the region corresponding to base pairs 135–152. The 5' start of the mRNA was mapped by primer extension with oligonucleotides (1) and (2) on RNA of adult worms. Oligonucleotides were kinased to a specific activity of 5,000 Ci mmol⁻¹ using [γ -³²P]ATP and T4-polymerase kinase, annealed with poly(A)⁺ RNA and extended by incubation with M-MLV transcriptase. Reaction products were analysed on a denaturing sequencing gel relative to an end-labelled *Hpa*II digest of pBR322, resulting in transcripts of 63 bases for primer (2) and 165 bases for primer (1). DNA sequencing of restriction fragments subcloned in M13 derivatives was by the chain termination method¹⁸ with both M13 and internal primers. Sequence data were confirmed in both directions, preferentially using the cDNA insert of independent λ isolates.

The primary structure of this antigen was confirmed by partial protein sequence analysis of the native 28K antigen eluted from SDS-polyacrylamide gels and purified to homogeneity by reverse-phase HPLC. A trypsin digest of this material and separation of the cleavage products resulted in a complex pattern of peaks (Fig. 2). The sequence of three of the tryptic fragments could be matched with the corresponding peptide regions present in the amino-acid sequence predicted from the cDNA.

From primer extension experiments (Fig. 1) on RNA of adult worms it could be deduced that the cap-site of the messenger was located only 14 nucleotides upstream of the first base as given in Fig. 1. It seems therefore very likely that translation is initiated from the first ATG-codon at nucleotide position 7. The presence of a potential cleavage site for a signal peptide can be deduced by comparison with the empirical rules¹⁴, which suggest cleavage after residue 27 (Ala). However, the expression in *E. coli* of the complete protein containing 211 amino acids (data not shown) gave a band on SDS-polyacrylamide gel electro-

phoresis (PAGE) gels which migrated at an identical position relative to the native protein. We therefore suggest that this particular antigen is not secreted by the organisms following the classical pathway described for many other surface antigens. It would appear that this 28K antigen of *S. mansoni* is not glycosylated, as no potential *N*-glycosylation sites are present in the predicted amino-acid sequence, confirming previous observations that the size of the *in vitro* translation product is identical to the size of the native protein. The frequency of this gene in the λgt10 and λgt11 libraries, 2×10^{-4} and 5×10^{-5} respectively, was certainly lower than expected assuming that this component represented around 1% of the proteins in total extracts of adult worms.

Experimental immunization

The method adopted for isolating this antigen presumably selected those parts of the mature 28K antigen which contained the major epitopes recognized by the host immune system. Compar-

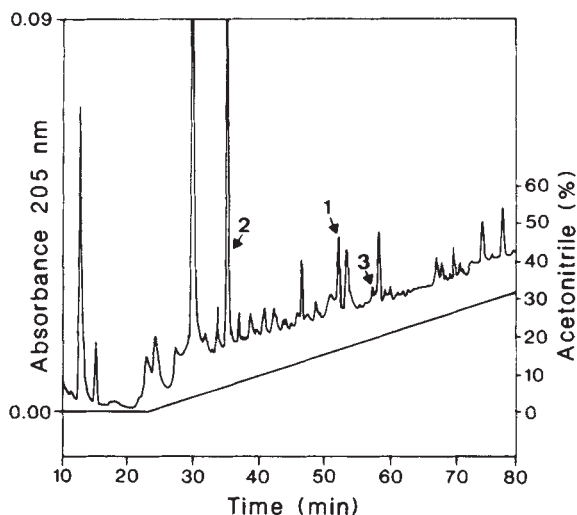


Fig. 2 Partial sequence analysis the native 28K antigen eluted from SDS-PAGE gels. Sequences of arrowed peptides as follows: 1, Leu-Pro-Ala-Val-Lys; 2, Tyr-Met-Ala-Lys; 3, Xxx-Asn-Leu-Leu-Ala-Xxx-Xxx-Pro-Xxx; Xxx is an unidentified residue.

Methods. Adult worms of *S. mansoni* were homogenized in phosphate-buffered saline (PBS) and soluble antigens were suspended on preparative SDS-PAGE gels. The fraction corresponding to a M_r of 28K was electro-eluted, dialysed against water and concentrated by lyophilization. The sample was made up in 20% in 1-propanol and purified by HPLC chromatography on a Vydac C18, 10 μ m column (reference 201 TP54, The Separations Group), equilibrated in 0.1% trifluoroacetic acid. The column was eluted with a 20–60% gradient of 1-propanol over 60 min and fractions containing the immunogenic 28K component were identified by Western blotting with a polyclonal rabbit antibody raised against the 28K gel fraction of adult worms. Protein (25 μ g) was lyophilized and resuspended in 8M urea, diluted five times in 0.1M *N*-ethyl morpholine acetate, 0.1 mM CaCl_2 , pH 8.3, containing 1.25 μ g of L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK)-trypsin (Worthington). Sample was digested for 4 h at 37 °C and the reaction was stopped by adding trifluoroacetic acid to 1%. After lyophilization, the material was resuspended in 0.1% trifluoroacetic acid and separated on a Vydac C18, 5 μ m column (reference 218TP54, The Separations Group), equilibrated in 0.1% trifluoroacetic acid and developed with a 0–60% gradient of acetonitrile in 120 min. Peptides labelled with a number in the chromatogram were loaded on a gas phase sequencer (model 470, Applied Biosystems) and phenylthiohydantoin amino acids were identified by on-line analysis (model 120, Applied Biosystems).

ing the position of the different cDNA inserts (Fig. 1), it can be concluded that the major antigenic epitopes in this molecule are located between amino-acid residues 91 and 200.

A recombinant antigen containing the essential part of the adult *S. mansoni* 28K antigen was expressed in *E. coli* as a cII fusion protein. Appropriate cDNAs originating from λ gt11 candidates were inserted directly, into a P_L -expression vector¹⁵ using the unique *Eco*RI site located 14 amino acids downstream of the *cII* initiation codon. This construction, containing the insert from λ TG06, directed the synthesis of a fusion protein with M_r 25K containing 172 amino acids of the C-terminal region of the native 28K antigen.

Cultures were induced for the synthesis of this fusion protein by incubation at 42 °C and extracts were analysed on Coomassie-Blue stained SDS-PAGE gels. A protein band with M_r 25K was observed, most of this material being present in the non-soluble fraction obtained after lysis of the cells by sonication. The

protein was recovered with a minimum purity of 80% by solubilization in the presence of 0.2% SDS, giving a solution of about 1 mg ml⁻¹ contaminated with small amounts of *E. coli* membrane protein components. A sample of this preparation was analysed by Western blotting (Fig. 3a), indicating that the fusion protein was efficiently recognized either by the rat or the rabbit antisera raised against the native antigen. The biological property of the 25K fusion protein was tested by experimental vaccinations of rats and hamsters using 50 μ g of the solubilized protein in the presence of aluminium hydroxide adjuvant. Animals received a second dose two weeks later by intraperitoneal injection and sera were collected starting from day eight. Rat samples were tested on Western blots for the presence of specific antibodies against the native 28K antigen of *S. mansoni* (Fig. 3b). The maximum response, observed after three weeks, reached levels comparable to those observed with sera raised against the native 28K protein. Note that extracts of the related species *Schistosoma bovis*, *S. japonicum* and *S. haematobium*, showed the presence in adult worm antigen of identical epitopes which were recognized by the rat serum against the fusion protein of M_r 25K (Fig. 4). Control immunizations with SDS-extracted membrane fractions of *E. coli* were always negative.

The biological activity of the recombinant antigen was determined in an *in vitro* antibody-dependent cellular cytotoxicity (ADCC) assay using normal rat eosinophils and sera¹⁶ from rats immunized either with the 25K fusion protein or a solubilized membrane fraction of *E. coli*. In particular samples taken at day 16 showed a high percentage (80%) of cytotoxicity towards schistosomula. Heat-inactivation or immunoglobulin E (IgE) depletion on a goat anti-IgE affinity column reduced the killing efficiency of these sera to 35%, indicating that the cytotoxicity was mainly due to IgE antibodies. The IgE antibody response to P28 was further confirmed by positive passive cutaneous anaphylaxis (PCA) reactions. Addition of a source of complement factors (guinea pig serum), which may have been inactivated during heat treatment, did not restore the levels of cytotoxicity to the original value. Finally, control rats injected with *E. coli* membrane fractions of comparable composition but lacking the cII fusion protein, showed no significant cytotoxicity in the ADCC assays. These encouraging results led us to attempt a challenge with live cercariae on a series of animals vaccinated with the 25K fusion protein produced in *E. coli*. Both a permissive (golden hamster) and semi-permissive (Fischer rats) host were chosen for this study.

Animals were immunized as described before by two injections with 50 μ g of the fusion protein. Six weeks after the second injection, rats and hamsters were infected percutaneously with 1,000 and 250 cercariae respectively. Rats were bled weekly after challenge infection and antisera were used to immunoprecipitate metabolically-labelled proteins of *S. mansoni* adult worms. The sera of infected control rats (Fig. 5, lanes 1 and 2) were not able to immunoprecipitate any antigens of the parasite (Fig. 5, lanes 1 and 2), but animals vaccinated with the recombinant fusion protein showed a strong antibody response against the 28K protein (Fig. 5, lanes 3, 4, 5). The evaluation of parasite burden in rats was performed 21 days after infection, just before the natural parasite rejection, and 30 days after the challenge for the hamster population. The results for both rat and hamster (Fig. 5) show a significant reduction in the number of parasites recovered from those populations which had been immunized with the fusion protein containing the major epitopes of this particular 28K adult worm antigen.

With the next series of immunization experiments we attempted to induce a comparable cell-mediated humoral response in monkeys (baboons). This cytotoxicity could be analysed *in vitro* by incubating the sera in the presence of purified human eosinophils. The sera from two baboons, vaccinated with two injections of either 100 μ g or 200 μ g of 25K M_r fusion protein, in the presence of aluminium hydroxide, induced

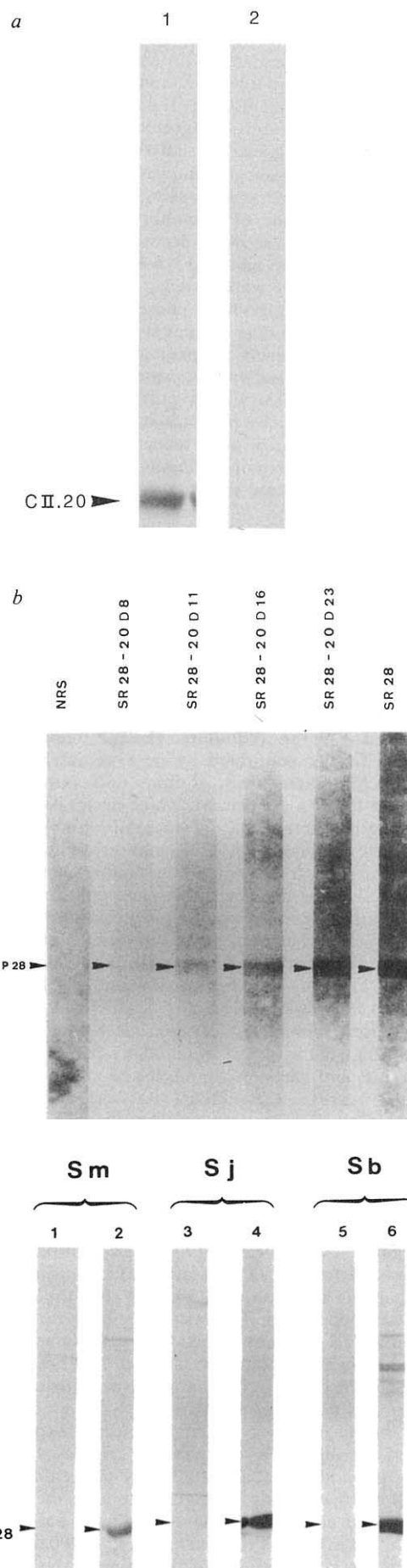
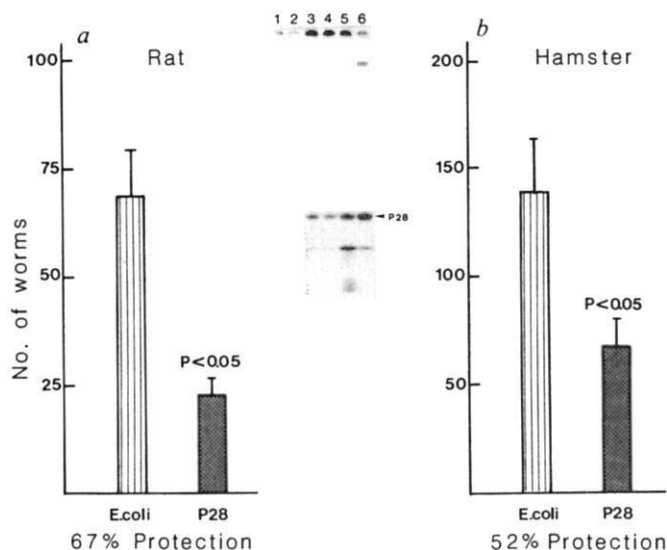


Fig. 3 *a*, Western blotting analysis¹⁹ of the 25K fusion protein. The fusion protein is strongly recognized by an anti-P28 rat serum obtained by immunizing animals with the P28 antigen purified from a crude extract of adult worm (lane 1). A preparation of *E. coli* membrane protein components was used as control (lane 2). CII.20 (arrowed), position of the 25K fusion protein containing 172 amino acids (20K) of the C-terminal region of the 28K antigen and a portion of the *cII* gene. *b*, Kinetic analysis of the humoral response after immunizing rats with the recombinant 28K protein. Western blotting and immunochemical reaction were used to follow the appearance of the antibody response against the 28K protein. Arrow, expected position of P28 antigen. We restricted this study to the IgG/IgM isotypes as markers of immunization of the vaccinated animal immune system. Recognition by immunized rat sera of the native 28K *S. mansoni* antigen occurred at day 11 after the second injection of recombinant antigen, and increased until day 23. SR28-20 represents rat serum against the CII.20 fusion protein obtained 8, 11, 16 and 23 days (D8-D23) after the second injection. SR28, rat serum directed against the native 28K antigen; NRS, normal rat serum.

Methods. Western blots were performed as described¹⁵. SDS-PAGE-fractionated native 28K antigen (~1 µg) was loaded on each lane of a 13% polyacrylamide slab gel and then transferred to nitrocellulose membrane in 20% methanol, 5 mM Tris-HCl, pH 6.8, 75 mM Glycine, for 2 h at 50 mA. Membranes were then saturated with 3% bovine serum albumin (BSA) in PBS and incubated with sera from vaccinated animals obtained 8, 11, 16 and 23 days after the second injection of the 25K fusion protein synthesized in *E. coli*. Rat serum directed against the native 28K antigen (SR28) was used as positive control, and normal rat serum (NRS) as negative control.

Fig. 4 Identification of epitopes commonly presented by the 28K fusion protein in extracts of related schistosome species. Adult worm homogenates (5 µg) from *S. mansoni* (Sm), *S. bovis* (Sb) and *S. japonicum* (Sj) were fractionated on 13% polyacrylamide slab gels, under denaturing and reducing conditions and electrophoretically transferred to nitrocellulose sheets. Lanes 1, 3, 5, sera from control rats immunized with *E. coli* lysate; lanes 2, 4, 6, sera from rats immunized with the fusion protein. Using SR28-20 D23 serum (Fig. 3), it was shown that the epitopes can equally be detected in these three species of schistosomes and are shared by a protein with M_r 28K. Recently, we have also identified this particular antigen in a crude homogenate from *S. haematobium* adult worm (data not shown). Arrowheads, position of 28K antigen.

Fig. 5 Vaccination experiments using the 25K fusion protein in rat (a) and hamster (b). Bars show number of parasites recovered from animals 21 days (rat) or 30 days (hamster) after challenge with cercariae: membrane fractions of *E. coli* (lined bars) were used as controls for the 28K fusion protein (dotted bars); *P* is the probability that the difference is due to chance alone. Inset in a, immunoprecipitation of rat serum. After immunization, the antibody response was allowed to return to levels at which no IgG/IgM specific antibodies were detected in the vaccinated rat sera (lane 2) compared to a normal rat serum (lane 1). A challenge infection was performed to exposing rats and hamsters percutaneously to 1,000 and 250 cercariae respectively. The evolution of the post-infection antibody response was analysed. Eight days after the challenge, IgG response directed towards the P28 antigen increased again and remained stable until the day of perfusion as analysed by immunoprecipitation of metabolically 35 S-methionine-labelled adult worm proteins (lane 1, normal rat sera; lane 2, infection day; lane 3, day 8 after infection; lane 4, day 15; lane 5, day 21; lane 6, anti-native 28K rat sera). In b, the parasite population recovered from hamsters was reduced by 52% ($P < 0.05$).



high levels of eosinophil-dependent cytotoxicity ($34.6 \pm 14.2\%$ and $95.3 \pm 5.6\%$ respectively). Heat-inactivation of these sera reduced the activity to 50% or less. These preliminary results indicated that in monkeys, as in the rat model, both IgG and IgE anaphylactic isotypes are induced by the 28K recombinant antigen. Under the same experimental conditions, naturally-infected baboon serum induced $48.5 \pm 10.6\%$ killing efficiency. Sera from control baboons, immunized with SDS-extracted membrane fractions of *E. coli*, induced no significant killing activity of the human eosinophils ($<10\%$). These results correlate with those obtained *in vitro* in the rat and are a basis for subsequent vaccination experiments in primates using more significant numbers of animals.

Conclusions

This paper reports the first successful cloning and expression of a protective antigen present in extracts of adult worms of the parasite *Schistosoma mansoni*. The approach based on screening λ gt11 cDNA libraries with specific antibodies proved to be very successful. The structure of this 28K protein is confirmed by partial protein sequence data on the trypsin-treated native antigen, indicating that this protein is indeed a major component of the M_r 28K gel fraction, used as antigen in previous experimental vaccination studies. The cDNA sequence reveals a primary structure of 211 amino acids corresponding to a protein of M_r 28K. The protein expressed in *E. coli* has the same electrophoretic mobility as the native antigen, indicating that the molecule is not extensively modified after translation. Computer-assisted analysis of the protein structure shows neither repetitive elements nor significant similarity with protein sequence data from other parasites.

Injection of a recombinant fusion protein, containing 172 amino acids of the C-terminal part of this 28K antigen, induces a strongly cytotoxic humoral response in rats, hamsters and baboons, composed of both IgE and IgG antibody classes. The first isotype was demonstrated to be a major factor responsible for the significant levels of eosinophil dependent cytotoxicity against schistosomula in vaccinated rats. This observation, although not excluding the function of IgG antibodies, supports the critical role of IgE response in the development of acquired protective immunity against infection by *S. mansoni* or other helminth parasites in general¹⁷.

In conclusion, we report the cloning and expression of a protective antigen which induces high level of antibody-mediated cytotoxicity in rodents or monkeys and in particular immunizes rats and hamsters against experimental infection with live cercariae. Experiments in progress will further test the protective properties of this antigen, which can then be considered as the basis for a vaccine protecting both humans and animals against schistosomiasis.

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- Harn, D. A., Mitsuyama, M. & David, J. R. *J. exp. Med.* **159**, 1371-1375 (1984).
- Smith, M. A., Clegg, J. A., Snary, D. & Trejdosiewicz, A. *J. Parasitology* **84**, 83-91 (1982).
- Smith, M. A. & Clegg, J. A. *Science* **227**, 535-538 (1985).
- Sher, A., Pearce, E., Hieny, S. & James, S. *J. Immun.* **136**, 3878-3883 (1986).
- Tarrab-Hazdai, R. et al. *J. Immun.* **135**, 2772-2779 (1986).
- Balloul, J. M., Pierce, R. J., Grzych, J. M. & Capron, A. *Molec. biochem. Parasit.* **17**, 105-114 (1985).
- Taylor, D. W., Cordingley, J. S. & Butterworth, A. E. *Molec. biochem. Parasit.* **10**, 305-318 (1984).
- Ianar, E., Pearce, E. J. & Sher, A. *Molec. biochem. Parasit.* **17**, 45 (1985).
- Harn, D. A., Mitsuyama, M., Huguene, E. D., Oligino, L. & David, R. J. *Molec. biochem. Parasit.* **16**, 345 (1985).

- Balloul, J. M., Grzych, J. M., Pierce, R. J. & Capron, A. *J. Immun.* (in the press).
- Huynh, T. V. in *DNA Cloning Techniques: A Practical Approach* (ed. Glover, D.) 49-78 (IRL Press, Oxford, 1984).
- Le Bouc, Y., Dreyer, D., Jaeger, F., Binoux, M. & Sondermeyer, P. *FEBS Lett.* **196**, 108-112 (1986).
- Lathe, R. & Lecocq, J. P. *Virology* **83**, 204-206 (1977).
- Von Heyne, G. *Eur. J. Biochem.* **133**, 17-21 (1983).
- Courtney, M. et al. *Proc. natn. Acad. Sci. U.S.A.* **81**, 669-673 (1984).
- Capron, M. et al. *Eur. J. Immun.* **8**, 127-133 (1978).
- Towbin, H., Staechlin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
- Capron, A. & Dessaint, J. P. *A. Rev. Immun.* **3**, 455-476 (1985).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).

Clathrin light chains contain brain-specific insertion sequences and a region of homology with intermediate filaments

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The primary structures of four bovine clathrin light chains have been determined. Light chains LC_a and LC_b are homologous proteins encoded by different genes. In the brain the messenger RNA from these genes undergoes differential splicing to yield proteins having centrally inserted brain-specific sequences. A potentially α -helical region of the clathrin light chains shows homology with intermediate filament proteins.

CLATHRIN is the major protein of the polyhedral coat of coated pits and vesicles¹. These membrane organelles are involved in various pathways of intracellular transport of which receptor-mediated endocytosis of macromolecules provides well-characterized examples^{2,3}. In contrast the role of clathrin-coated organelles in exocytic pathways of secretion is less well defined⁴⁻⁶.

During receptor-mediated endocytosis clathrin is assembled and disassembled in a regulated fashion^{2,7}. Clathrin coats are formed from trimeric molecules called triskelions consisting of three heavy chains of relative molecular mass 180,000 (M_r 180K) and three light chains of M_r 30–40K^{8,9}. Triskelions have a characteristic three-armed structure in which each arm consists of one heavy chain and one light chain. There is growing evidence that the heavy chain plays a structural role and that the light chains, located in the triskelion vertex^{10,11} are regulatory elements in clathrin function. For example, the light chains provide binding sites for calmodulin^{12,13} and an ATP-dependent uncoating protein that disassembles clathrin baskets¹⁴. In addition there are at least four distinct forms of clathrin light chain that may serve to introduce diversity into the structure and function of coated vesicles in mammalian tissues. Each cell has two distinct light chains, designated LC_a and LC_b. In brain both light chains are of higher apparent M_r than those isolated from other tissues¹⁵⁻¹⁸. In this paper we describe the structural and genetic basis for the differences between these four forms of clathrin light chains. The accompanying paper¹⁹ localizes different functions in two structurally distinct regions of the light chains.

Primary structure of clathrin light chains

Partial protein sequences were determined for LC_a and LC_b isolated from bovine brain and adrenal gland, a convenient source of non-brain clathrin¹⁸. Coated vesicles or clathrin triskelions were purified as described by Pearse and Robinson²⁰, boiled and then centrifuged to give a supernatant that contains clathrin light chains as major components^{12,18}. These were further purified by DEAE-cellulose chromatography²¹ or by C3 reverse phase (RP) HPLC. Amino-acid sequence analysis of LC_a and LC_b from both tissues indicated that the amino termini of the mature proteins were blocked. Light chains were then cleaved by chemical and enzymatic methods. Peptides produced were purified on C3 and C18 RP-HPLC columns¹⁸ and their

amino-acid sequences determined on a gas-phase sequencer. A total of 153 amino-acid residues for brain LC_a, 168 for brain LC_b, 75 for adrenal LC_a and 67 for adrenal LC_b were obtained. From overlapping peptides and the apparent homology between all four light chains, we were able to align almost all of these protein sequences (Fig. 2).

Five oligonucleotides were designed from the protein sequences and used to screen λ gt10 complementary DNA libraries made from bovine brain and from a bovine lymphocyte cell line (BL-3). This cell line was a convenient source of messenger RNA for the non-brain light chains²². Previous comparison showed that light chains from lymphocyte, adrenal, liver and other 'non-brain' tissues were indistinguishable¹⁶, indicating that lymphocyte cDNAs could be identified with oligonucleotides derived from adrenal protein sequence. The initial screen of 2.5×10^5 recombinants from both libraries was made with one oligonucleotide specific for LC_b and another specific for LC_a. Positive clones were then rescreened with all five oligonucleotides and only those clones hybridizing with two or more were selected for further study. The oligonucleotide screen yielded cDNA clones for lymphocyte LC_a and for lymphocyte and brain LC_b. No clones corresponding to brain LC_a were obtained although one clone corresponding to the non-brain form of LC_a was obtained from the brain library. This was not unexpected as brain contains significant amounts of non-brain light chains¹⁸. On rescreening the brain cDNA library with the lymphocyte cDNA clone, twenty more clones were obtained. Characterization of three clones revealed one that coded for brain LC_a.

The largest clones that code for each of the four light chains have inserts of 1.1–1.2 kilobases (kb), corresponding to the size of the mRNA as determined by Northern blot analysis. A single, major band of mRNA for each light chain was observed, which was slightly larger in brain than in adrenal gland or the lymphocyte cell line (data not shown).

Southern blot analysis²³ revealed distinct patterns of hybridization with the cDNA probes for LC_a and LC_b (data not shown). This showed that LC_a and LC_b are encoded by distinct genes. For LC_b the patterns of hybridization with digests using eight different restriction enzymes were simple and consistent with a single LC_b gene or multiple closely linked genes. For LC_a the patterns were complex and the results do not allow us to estimate the number of LC_a genes or their patterns of genetic linkage. However the sequence analysis described below provides no evidence for more than one LC_a or LC_b gene.

The nucleotide sequences of the four full-length cDNA clones are shown in Fig. 2a–d. The protein sequences predicted from the cDNA sequences are entirely consistent with those obtained by direct analysis (Fig. 2). In particular it should be noted that

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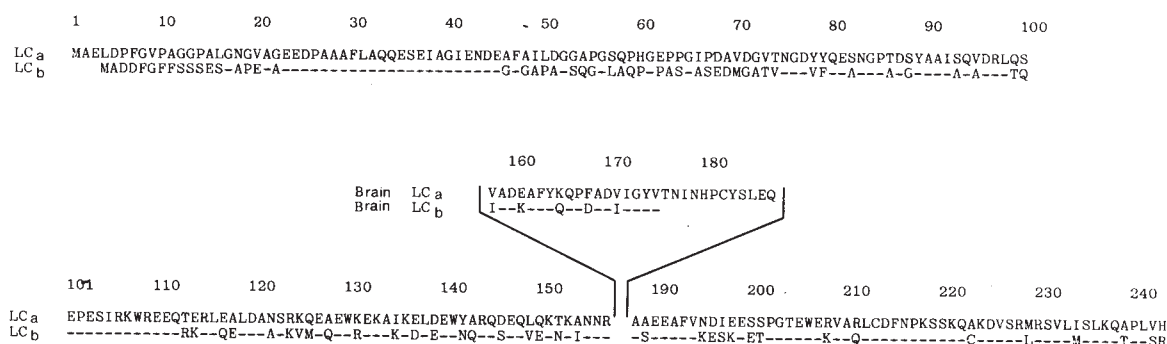


Fig. 1 A comparison of the amino-acid sequences of the four clathrin light chains. Numbering refers to the brain LC_a sequence. Amino-acid identities are denoted by a dash, differences are as indicated. The brain-specific inserts are projected. At position 14 in the lymphocyte LC_a sequence an H residue was found, differing from the P residue shown in brain LC_a. This probably results from allelic polymorphism (see text).

the protein sequences derived from adrenal light chains agree completely with cDNA sequences from the lymphocyte cell line.

The cDNA clones encompass the entire coding regions for all four clathrin light chains as judged by the following criteria: (1) all peptide sequences previously obtained are accounted for by the cDNA sequences; (2) the amino-acid compositions predicted from the cDNA sequences agree completely with experimentally determined compositions; (3) for lymphocyte LC_b a stop codon in-frame with the coding sequence is found 136 base pairs (bp) upstream from the initiator methionine; and (4) the cDNA clones and the mRNA are of comparable size. This was important to establish because the predicted M_r s of all the light chains (non-brain LC_b, 23.1K; non-brain LC_a, 23.3K; brain LC_b, 25.1K and brain LC_a, 26.7K) are 30% less than those determined by gel electrophoresis¹⁶. The anomalous electrophoretic behaviour was found to be a general property of the light chains and their fragments and probably reflects reduced SDS binding.

Differential splicing of brain light-chain mRNA

When the sequences of the four clathrin light chains are aligned (Fig. 1) the structural basis for their polymorphism becomes clear. Comparison of the non-brain forms of LC_a and LC_b shows they have distinct but homologous sequences. Non-brain LC_a is three amino acids longer than non-brain LC_b, correlating with its lower mobility on SDS-gel electrophoresis. There are amino-acid substitutions throughout the non-brain light-chain sequence consistent with LC_a and LC_b sharing a limited number of tryptic peptides^{17,18}. In contrast the brain forms of each light chain are identical to their non-brain counterparts except for the presence of inserted sequences at homologous positions about two-thirds of the way from the amino terminus to the carboxy terminus. This result is totally consistent with the similarity in the peptide maps of light chains obtained from brain and adrenal gland¹⁸. The 18-residue insertion in brain LC_b is homologous to the amino-terminal portion of the 30-residue insertion in brain LC_a. The presence of these inserted sequences explains why brain light chains have lower mobility on SDS gels than the non-brain forms. The increased size of the brain-specific insert in LC_a in addition to the extra three residues at the amino terminus accounts for the lower mobility of brain LC_a compared with brain LC_b.

The unusual pattern of amino-acid sequence identity between the brain and non-brain forms of each light chain is reflected in the nucleotide sequences of their corresponding mRNAs. Except for two point substitutions (discussed below) there is no divergence in the 5' and 3' untranslated regions or in the homologous segments of the coding regions. The critical difference is the insertion in the mRNAs encoding the brain forms of either a 54-bp (LC_b) or 90-bp (LC_a) segment encoding the brain-specific sequences.

Even when two non-allelic genes encode identical polypeptides, such as the two human α -globin genes, the nucleotide

sequence is conserved in the coding regions but diverges significantly in the 3' untranslated regions²⁴. The total conservation of flanking sequences between the brain and non-brain light chain mRNAs thus indicates that both are initially transcribed from the same genomic sequence.

In addition to the 90-bp insertion sequence there are two single base differences between the cDNA sequences for brain and lymphocyte LC_a. One is a C-to-A substitution that produces a change from histidine to proline at position 14 in the coding region. The other is a T-to-C substitution in the 3' untranslated region (Fig. 2a,b). These observations might appear to be inconsistent with the interpretation that LC_a mRNAs in these two tissues derive from a single gene. However, these substitutions probably represent allelic differences between the individual cows from which the brain and lymphocyte libraries were made. It is also possible that they arose from errors introduced by reverse transcriptase in the construction of the libraries²⁵.

If mRNAs for brain and non-brain forms of clathrin light chains are the products of single LC_a and LC_b genes, then their differences must result from differential splicing of the initial RNA transcripts. The additional sequences in the brain forms would then result from the expression of exons that are spliced out in other tissues. Differences in the sequences of the cDNA clones obtained from brain and the lymphocyte cell line are also seen at the poly(A) addition site. The lymphocyte cDNA for LC_b has an additional two guanine residues (CTTGGAA compared to CTCTAA) and the lymphocyte cDNA for LC_a has an additional AAAT sequence (AAGAAATAA compared to AAGAA) (Fig. 2a-d). This indicates that the processing of light-chain mRNA follows quite a different pathway in brain compared to other tissues.

Two patterns of tissue-specific splicing have been found to generate alternative forms of fibronectin²⁶: exon skipping, in which entire exons are spliced out of the mRNA, and exon subdivision, in which alternative splice acceptor sites in an exon are used. For LC_a the latter mechanism might be employed because of the presence of a canonical splice acceptor site at the 3' end of the brain-specific insertion sequence. In the case of LC_b no such site is present indicating that an exon skipping mechanism operates. A short region of sequence homology exists between the first eight residues of the brain-specific insert and the first eight residues on the carboxy-terminal side of the insert (Fig. 1). This may result from the existence of regulatory sequences that are involved in the splicing reactions or from the requirements for a particular feature of protein structure at this point in the sequence.

Conserved and variable regions

Alignment of the amino-acid sequence for LC_a and LC_b (Fig. 1) reveals 60% identity indicating that the LC_a and LC_b genes diverged from a common ancestor that underwent duplication. However, the light-chain sequences have not been uniformly

a

1 TTGCTCTGATAGCAGGCGCAAGCACAGCGCGCTGCTGGAGTTGGTGTGTTGCTGGTCGGTGGTGTCTCTGAAAGTCGGTGCCGCT 90

91 GAACCTCCCGCCATGGCTGAGTTAGATCCGTTCCGGCTCCCGCTGGCGGTCCCGCTGGGGAACGGAGTGGCGCGGCAAGAGGATCCT 180

M A E L D P F G V P A G G P A L G N G V A G E E D P -

181 GCGCGGCTCTTTGGCGCAGCAGGAGAGGAGATTGCGGGCATAGAGAACGACGAGGCTTCGCCATCTGGACGGCGCGCCCCCGGA 270

A A A F L A Q Q E S E I A G I E N D E A F A I L D G G A P G -

271 TCCAGCCTCAGCGGAGCGCGGGGATTCCAGATGCTGTTGATGGAGTGACGAATGGAGACTACTACCAGGAGAGTAATGGCGGACA 360

S Q P H G E P P G I P D A V D G V T N G D Y Y Q E S N G P T -

361 GACAGTTATGCAGCTATTTACAGTGGATCGATTGCAGTCAGAGCTGAAAGTATCCGTAATGGAGAGAAGAGCAACGGAACGCTTG 450

D S Y A A I S Q V D R L Q S E P E S I R K W R E E Q T E R L -

451 GAAGCCCTTGATGCCAATTTCTCGGAAGCAGGAAGCGGAGTGGAAAGAAAAGCAATAAGGAGCTGGACGAGTGGTATGCGAGGCAAGAT 540

E A L D A N S R K Q E A E W K E K A I K E L D E W Y A R Q D -

541 GAGCAGCTACAGAAGCAAAAGCCAACACAGGGTGGCAGATGAAGCTTTCTACAAACAACCTTCGCTGACGTGATTGGTTATGTCACA 630

E Q L Q K T K A N N R V A D E A F Y K Q P F A D V I G Y V T -

631 AACATAACCATCCTTGCTACAGCCTAGAACAGGCAGCAGAAGAAGCCTTTGTAAATGACATTGAGGAGTCGTCCCGAGGCACTGAGTGG 720

N I N H P C Y S L E Q A A E E A F V N D I E E S S P G T E W -

721 GAACGGGTGGCGCGCTGTGTGACTTTAACCCCAAGTCCAGCAAGCAGGCCAAAGATGTCTCCCGCATGCGTTCTGTCTCATCTCCCTC 810

E R V A R L C D F N P K S S K Q A K D V S R M R S V L I S L -

811 AAGCAGGCCCGCTGGTGCATGAAGAGCGCGCTGTGGAACACTACATCTGCAATATCTTAATCTACTCAGTGAAGCTCTTCACGGT 900

K Q A P L V H *

901 AATTGGATTAATTATGTTAGTCTTTTGGACCAACCTTTTGTCTTTAGAGTTGATCATTGTTTGTGATTGCATGTTTCCTTCAACTGT 990

GTCTCCCTGGCATTCAAAGAGGAGGGAGGTGGCGGCAGAGGAAGGAGGAGGCTCCCAACGGCAGCCTCAACCTATGCTTTTGTGCTTCTGTC 991

ATTATTCTGAGATAAATTCTGTTCCAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAG 1080

1081 ----- 1158

b

1 CAAGCACAGCGCGCTGCTGGAGTTGGTGTGTTGCTGGTCGGTGGTGTCTCTGAAAGTCGGTGCCGTCGAACCTCCCGCCATGGGCTG 90

M A E -

91 AGTTAGATCCGTTCCGGCTCCCGCTGGCGGTACGCCCTGGGGAACGGAGTGGCGCGGCAAGAGGATCCTGCCCGCGCTCTTGGCGC 180

L D P F G V P A G G H A L G N G V A G E E D P A A A F L A Q -

181 AGCAGGAGAGCAGATTGCGGGCATAGAGAACGACGAGGCTTCGCCATCTGGACGGCGCGCCCCGGATCCAGCCTCAGCGCAGC 270

Q E S E I A G I E N D E A F A I L D G G A P G S Q P H G E P -

271 CGCGGGGATTCCAGATGCTGTTGATGGAGTGACGAATGGAGACTACTACCAGGAGAGTAATGGCGGACAGACAGTTATGCAGTATT 360

P G I P D A V D G V T N G D Y Y Q E S N G P T D S Y A A I S -

361 CACAAGTGGATCGATTGCAGTCAGAGCTGAAAGTATCCGTAATGGAGAGAAGAGCAACGGAACGCTTGAAGCCCTTGATGCCAATT 450

Q V D R L Q S E P E S I R K W R E E Q T E R L E A L D A N S -

451 CTGGAAGCAGGAAGCGGAGTGGAAAGAAAAGCAATAAGGAGCTGGACGAGTGGTATGCGAGGAGGATGAGCAGCTACAGAAGACAA 540

R K Q E A E W K E K A I K E L D E W Y A R Q D E Q L Q K T K -

541 AAGCCAACAACAGGCGCAGAGAAGCCTTTGTAAATGACATTGAGGAGTCGTCCCGAGGCACTGAGTGGGAACGGGTGGCGCGGCTGT 630

A N N R A A E E A F V N D I E E S S P G T E W E R V A R L C -

631 GTGACTTTAACCCCAAGTCCAGCAAGCAGGCCAAAGATGTCTCCCGCATGCGTTCTGTCTCATCTCCCTCAAGCAGGCCCGCTGGTGC 720

D F N P K S S K Q A K D V S R M R S V L I S L K Q A P L V H -

721 ACTGAAGAGCGCGCTGTGGAACACTACATCTGCAATATCTTAATCTACTCAGTGAAGCTCTTACGGTAATTGGATTAATTATGTTG 810

*

811 AGTTCTTTTGGACCAACCTTTTGTCTTTAGAGTTGATCATTGTTTGTGATTGCATGTTTCCTTCAACTGTGTTCTCCCTGGCATTCAAA 900

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TCTGTCCAAGAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1046

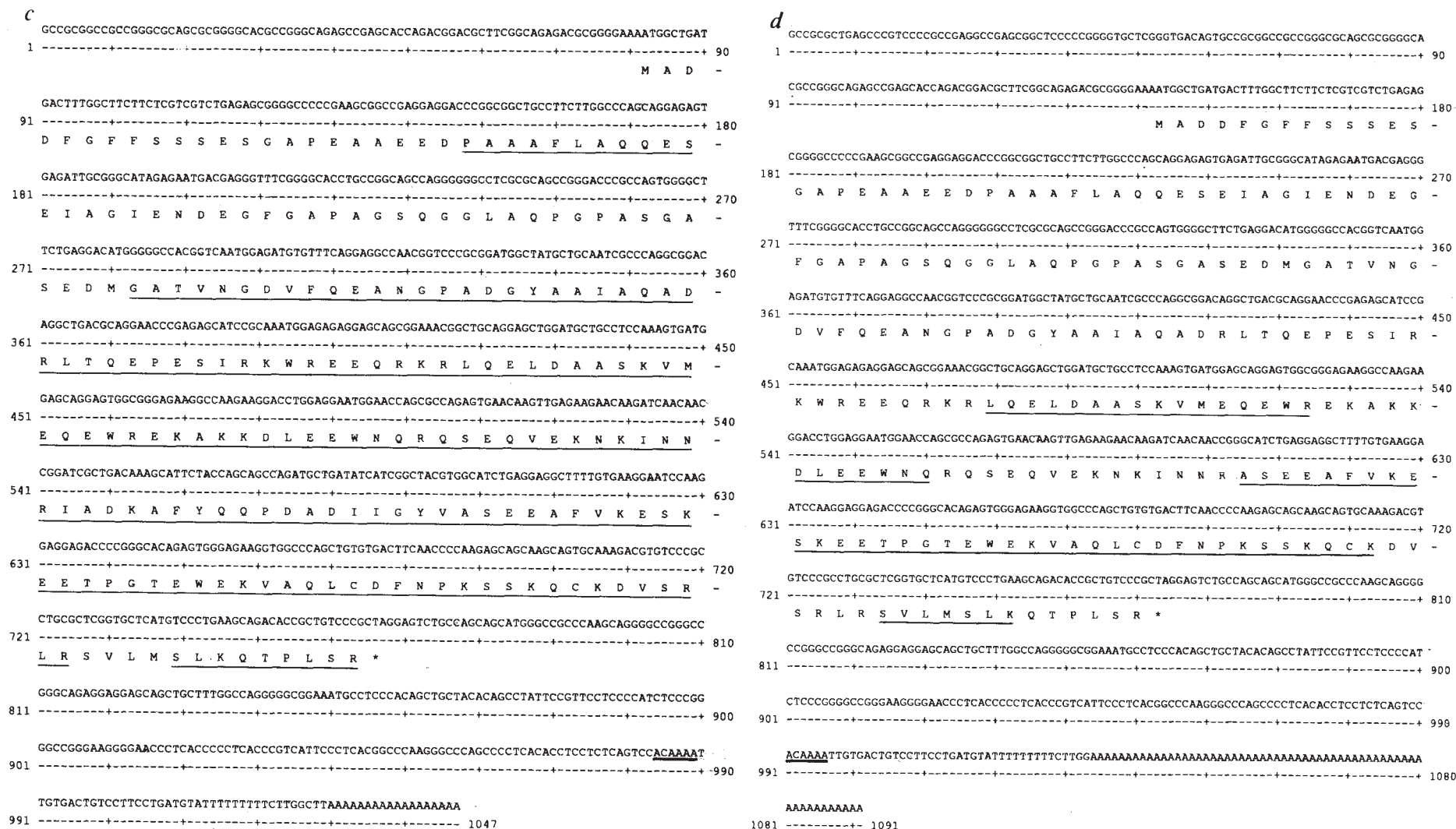


Fig. 2 The nucleotide and predicted amino-acid sequences of *a*, brain LC_a; *b*, lymphocyte LC_a; *c*, brain LC_b and *d*, lymphocyte LC_b. The two nucleotide differences between brain and non-brain LC_a outside the tissue-specific insert are underlined. Putative polyadenylation signals are double underlined³⁸. The underlined amino-acid sequences were independently confirmed by means of peptide sequencing.

Methods. Full length cDNA clones were subcloned in both orientations into the *EcoRI* site of M13mp18. Single-stranded DNA was prepared and sequenced by the dideoxy method using [α -³⁵S]dATP³⁹. The complete sequence on both strands was obtained by subcloning and sequencing restriction enzyme fragments and by the use of oligonucleotides specific for internal sequences⁴⁰. Sequences were assembled and analysed using computer programs of Staden⁴¹ and the University of Wisconsin⁴².

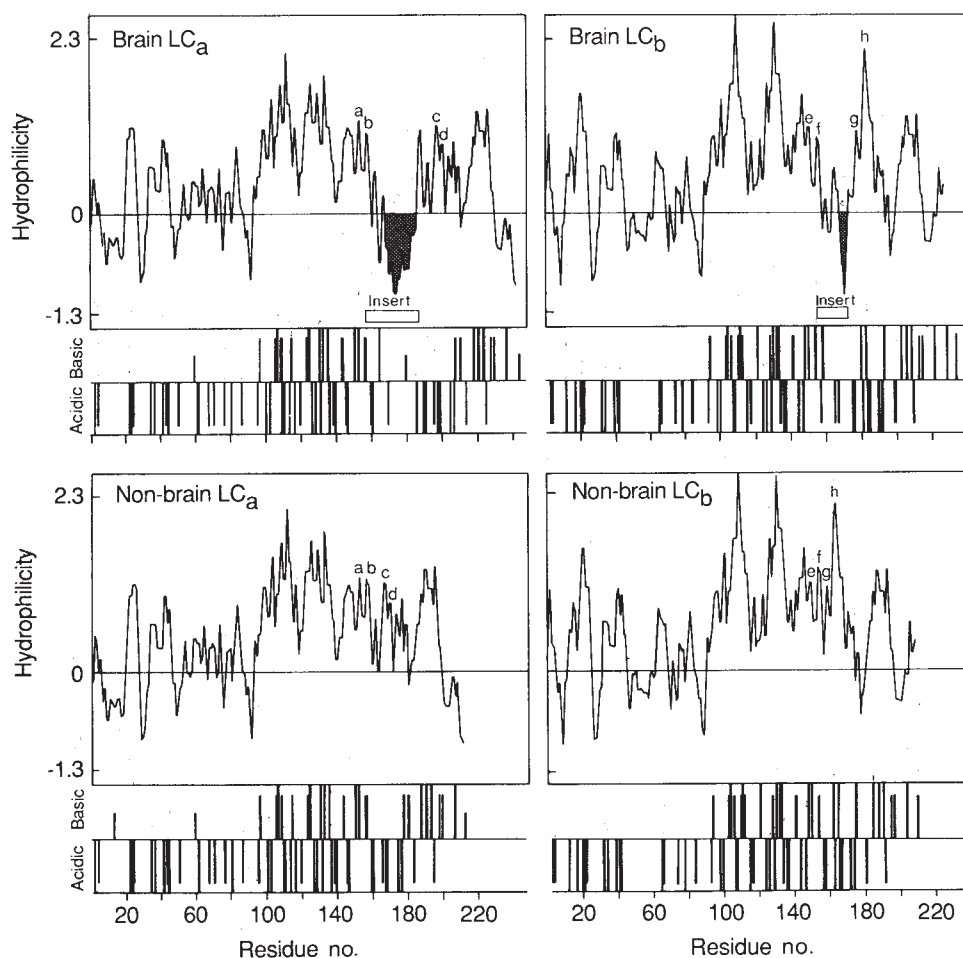


Fig. 3 Hydrophilicity plot and charge distribution for clathrin light chains. The plots record the average hydrophilicity along the sequences over a window of nine residues³⁰. Hydrophilic and hydrophobic residues are in the upper and lower part of the frame respectively. Peaks that flank the brain-specific insertion sequences and are shared by the brain and non-brain forms of LC_a and LC_b are marked a, b, c, d and e, f, g, h respectively. Below each panel is the distribution of charged residues with basic residues above the line with histidines represented by the shortest lines, lysines by the longest and arginine by lines of intermediate length; and acidic residues below the line, with aspartic acid as the shorter and glutamic acid as the longer lines.

conserved. A striking feature is at the amino-terminal end of the proteins. An uninterrupted block of 23 conserved amino acids is flanked by two stretches of roughly 20 amino acids which represent the most divergent portions of the molecules. In the amino-terminal diversity region of LC_b there are four serine residues in a stretch of five amino acids which are not found in LC_a. One or more of these residues may be the site of phosphorylation that only occurs in LC_b²⁷⁻²⁹. In contrast to the amino-terminal region, the distribution of conserved residues is more uniform in the carboxy-terminal 60% of the polypeptides.

A clear differentiation in the distribution of charged and hydrophobic amino acids between the amino-terminal 40% and carboxy-terminal 60% of the molecule is also apparent (Fig. 3). Although there are no large hydrophobic segments in either portion of the molecule, the charged residues are largely confined to the carboxy-terminal region. More strikingly, the overall acidic character of the light chains reflects the fact that virtually all the charged residues in the amino-terminal 40% of the molecule are aspartic and glutamic acids. These observations emphasize the chemically distinct nature of the two regions of the light chains which is correlated with the antigenic and functional differences described in the accompanying paper¹⁹.

A comparison of the hydrophilicity plots³⁰ for both LC_a and LC_b shows that brain-specific sequences introduce a prominent hydrophobic peak that is not found in the lymphocyte forms (Fig. 3). In coated vesicles the brain-specific sequences are exposed and these hydrophobic elements may therefore interact with molecules or organelles of the brain cytoplasm¹⁹.

All species of higher vertebrates examined show two classes of clathrin light chains that presumably correspond to LC_a and LC_b. In contrast, yeast expresses a single light chain⁵. The persistence of two light-chain classes in the evolution of higher

eukaryotes, their expression in all tissues and their significant differences in structure all point to the conclusions that the two light chains are functionally differentiated and that both are required for clathrin function.

Homology with intermediate filaments

A computer search of the NBRF (National Biomedical Research Foundation) protein databank with the program FASTP³¹ revealed an interesting similarity between the central region of the clathrin light chain and the α -helical domain of various intermediate filament proteins, particularly the cytokeratins³². This similarity (Fig. 4) was judged to be of statistical significance by the program RDF³¹. Further support for the significance of the homology is the observation that residues which are highly conserved between intermediate filament proteins tend to be those that are also shared with the clathrin light chains (residues marked with a triangle in Fig. 4).

Homology between clathrin light chains and intermediate filament proteins has two major implications. First, the amino-acid sequence similarities suggest that the structures of the homologous segments may be similar. Specifically, it is known that the segment of the intermediate filament proteins marked 'coil' in Fig. 4 forms α -helices which are involved in the coiled coil structure characteristic of the intermediate filaments³³. Predictive analysis of this portion of the light-chain sequences by two independent methods indicates a high helix-forming potential for this part of the protein^{34,35}. However, it is unlikely that this helix would be involved in a coiled coil structure such as is found in intermediate filaments. Sequences forming a coiled coil have a pattern of hydrophobic amino acids that are spaced alternatively at 3 and 4 residues³⁶. These positions in the intermediate filament proteins are designated with a filled circle in

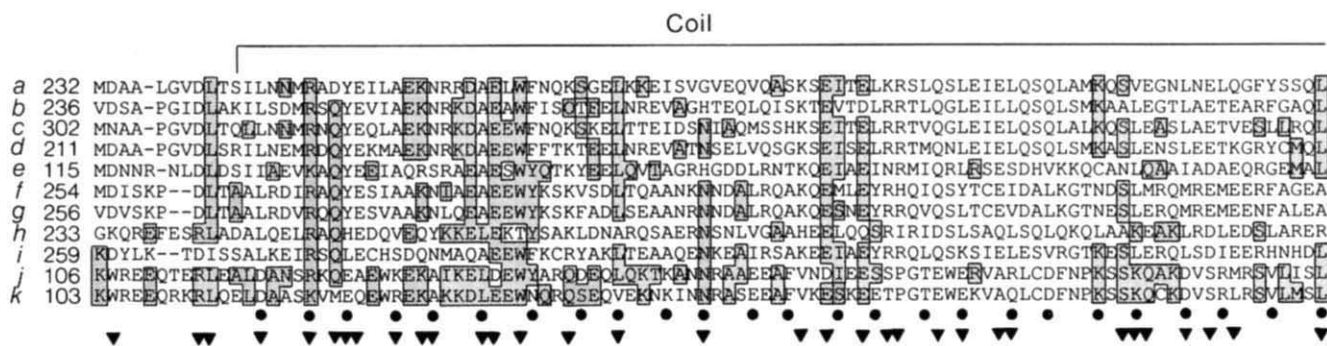


Fig. 4 Homology between the central region of each non-brain clathrin light chain (residues 107–236) and the α -helical domain of intermediate filaments. Amino-acid identities are boxed. ∇ , Positions in the intermediate filaments where amino acids have been conserved in seven out of nine proteins; $\bullet$, positions in the intermediate filaments of hydrophobic residues comprising the 3/4 periodicity which contributes to the formation of coiled coil structures³⁶. The sequences are of the following proteins: *a*, *Xenopus laevis* embryonal cytokeratin⁴³; *b*, bovine type I cytokeratin⁴³; *c*, mouse type I cytokeratin⁴⁴; *d*, human type I cytokeratin³³; *e*, human type II cytokeratin³³; *f*, chicken desmin³³; *g*, hamster vimentin³³; *h*, human nuclear lamin⁴⁵; *i*, porcine neurofilament³³; *j*, clathrin LC_a; *k*, clathrin LC_b.

Fig. 4. In the light-chain sequences there is little conservation of hydrophobicity at these positions. The prediction that the light chains will not form coiled coils is consistent with Ungewickell's observations that free light chains do not form oligomers²¹. In the accompanying paper, we show that the region of homology with intermediate filaments is involved in interaction with the clathrin heavy chain¹⁹.

The similarity shown in Fig. 4 suggests that the intermediate filament proteins and the clathrin light chains are evolutionarily related to each other. The finding that homology is restricted to only one segment of each protein is not uncommon and is believed to result from the shuffling of exons by recombination³⁷. Current analysis of the structure of the light-chain genes will reveal whether the region of homology with intermediate filaments is encoded by a separate exon. If so, then the introduction of this exon during the evolution of the light-chain genes may have provided the structure necessary for binding to the heavy chain.

Conclusions

These studies have determined the structural basis for the four polypeptide forms of clathrin light chain so far identified. None

of the differences is trivial and the results strongly indicate that considerable functional diversification of the clathrin light chains has occurred in eukaryotic evolution. Thus we believe that all four light chains will be shown to have both common and unique functions. The specialization between LC_a and LC_b may be understood by comparison of the function of clathrin in yeast and in mammalian cells. Explaining the remarkable differences between the brain and non-brain forms of the clathrin light chains will clearly require further definition of the functions of coated vesicles in different tissues. In the accompanying paper we relate the structure of the clathrin light chains to their interactions in coated vesicle function¹⁹.

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- Pearse, B. M. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1255–1259 (1976).
- Pearse, B. M. F. & Bretscher, M. B. A. *Rev. Biochem.* **50**, 85–101 (1981).
- Goldstein, J. L., Brown, M. S., Anderson, R. G. W., Russell, D. W. & Schneider, W. J. A. *Rev. Cell Biol.* **1**, 1–39 (1985).
- Rothman, J. E. *Nature* **319**, 96–97 (1986).
- Payne, G. S. & Schekman, M. *Science* **230**, 1009–1014 (1985).
- Kelly, R. B. *Science* **230**, 25–32 (1985).
- Hanover, J. A., Willingham, M. C. & Pastan, I. *Cell* **39**, 283–293 (1984).
- Ungewickell, E. & Branton, D. *Nature* **289**, 420–422 (1981).
- Kirchhausen, T. & Harrison, S. C. *Cell* **23**, 755–761 (1981).
- Ungewickell, E., Unanue, E. R. & Branton, D. *Cold Spring Harb. Sym. quant. Biol.* **46**, 723–731 (1982).
- Kirchhausen, T., Harrison, S. C., Parham, P. & Brodsky, F. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2481–2485 (1983).
- Lisanti, M. P. *et al. Eur. J. Biochem.* **125**, 463–470 (1982).
- Linden, C. D. *Biochem. biophys. Res. Commun.* **109**, 186–193 (1982).
- Schmid, S. L., Braell, W. A., Schlossman, D. M. & Rothman, J. E. *Nature* **311**, 228–231 (1984).
- Pearse, B. M. F. *J. molec. Biol.* **126**, 803–812 (1978).
- Brodsky, F. M. & Parham, P. *J. molec. Biol.* **167**, 197–204 (1983).
- Creutz, C. E. & Harrison, J. R. *Nature* **308**, 208–210 (1984).
- Holmes, N., Biermann, S. J., Brodsky, F. M., Bharucha, D. & Parham, P. *EMBO J.* **3**, 1621–1627 (1984).
- Brodsky, F. M. *et al. Nature* **326**, 203–205 (1987).
- Pearse, B. M. F. & Robinson, M. S. *EMBO J.* **3**, 1951–1957 (1984).

- Ungewickell, E. *EMBO J.* **2**, 1401–1408 (1983).
- Brodsky, F. M. *J. Cell Biol.* **101**, 2055–2062 (1985).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Michelson, A. M. & Orkin, S. H. *Cell* **22**, 371–377 (1980).
- Battula, N. & Loeb, L. A. *J. biol. Chem.* **249**, 4048–4093 (1974).
- Hynes, R. A. *Rev. Cell Biol.* **1**, 67–90 (1985).
- Usami, M., Takahashi, A., Kadota, T. & Kadota, K. *J. Biochem.* **97**, 1819–1822 (1985).
- Schook, W. J. & Puskin, S. *Proc. natn. Acad. Sci. U.S.A.* **82**, 8039–8043 (1985).
- Bar-zvi, D. & Branton, D. *J. biol. Chem.* **261**, 9614–9621 (1986).
- Hopp, T. P. & Woods, K. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3824–3828 (1981).
- Lipman, D. J. & Pearson, W. R. *Science* **227**, 1435–1441 (1985).
- Lazarides, E. A. *Rev. Biochem.* **51**, 219–250 (1982).
- Geisler, N., Fischer, S., Vandekerckhove, J., Plessmann, U. & Weber, K. *EMBO J.* **3**, 2701–2706 (1984).
- Chou, P. Y. & Fasman, G. D. *Adv. Enzymol.* **47**, 45–148 (1978).
- Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97–120 (1978).
- McLachlan, A. D., Stewart, M. & Smillie, L. B. *J. molec. Biol.* **98**, 281–291 (1975).
- Rogers, J. *Nature* **315**, 458–459 (1985).
- Wickens, M. & Stephenson, P. *Science* **226**, 1045–1051 (1984).
- Biggin, M. D., Gibson, T. J. & Hong, G. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3936–3965 (1983).
- Strauss, E. C., Kohori, J. A., Siu, G. & Hood, L. E. *Analyt. Biochem.* **154**, 353–360 (1986).
- Staden, R. *Nucleic Acids Res.* **12**, 499–503 (1984).
- Devereux, J., Haeblerli, P. & Smithies, O. *Nucleic Acids Res.* **12**, 387–395 (1984).
- Bader, B. L., Magin, T. M., Hatzfeld, M. & Franke, W. W. *EMBO J.* **5**, 1865–1875 (1986).
- Steinert, P. M., Rice, R. H., Roop, D. R., Trus, B. L. & Steven, A. C. *Nature* **302**, 794–800 (1983).
- McKeon, F. D., Kirschner, M. W. & Caput, D. *Nature* **319**, 463–468 (1986).

Interstellar diamonds in meteorites

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Primitive meteorites contain up to 400 p.p.m. of a very fine-grained type of carbon, noncommittally called C δ ¹. It apparently comes from outside the Solar System, as it carries isotopically anomalous krypton and xenon ('Xe-HL' or 'CCFXe', enriched twofold in the lightest and heaviest isotopes²) and nitrogen ($\delta^{15}\text{N} = -330\%$ ³; that is, depleted in ^{15}N by -330% relative to atmospheric nitrogen), although the carbon itself is within the terrestrial range ($\delta^{13}\text{C} = -38\%$ ¹). Expanding on a preliminary report⁴, we now present evidence that part or all of C δ is diamond—not shock-produced but primary, formed by stellar condensation as a metastable phase. It appears that interstellar dust contains diamond.

Thinking that C δ might be the C₆₀ cluster 'buckminsterfullerene'⁵, we isolated a new sample from the Allende C3V chondrite, aiming for maximum purity rather than maximum yield. After dissolving the major minerals in HF-HCl, we generated a colloid from the remaining 0.5% residue, leaving behind coarse-grained spinel and chromite^{2,6}. The major gas-poor part (C γ) of the colloidal carbon was removed by oxidation with 16 M and 21 M HNO₃ (16 h, 70 °C) and concentrated HClO₄ (2 h, 140 °C). Surprisingly, the residue (Allende CH, 109 p.p.m.) was not black but light tan. As it contained some silicate contaminants, we reprocessed it with HCl and HF, obtaining Allende CJ (89 p.p.m.) of unchanged colour.

On the scanning electron microscope (SEM) Allende CJ showed no elements above oxygen (Mg, Al and Si <2.5 wt% if evenly dispersed or <1% if in grains of >0.5 μm). X-ray diffraction showed only three very broad, diffuse lines, which to our surprise matched the three principal lines of diamond. On the transmission electron microscope (TEM), the grains turned out to be typically 50 Å in size (Fig. 1). Electron diffraction (Fig. 2) confirmed that most grains were diamond, as shown by the agreement of the observed spacings with accepted values (shown in parentheses): 2.06 (2.06), 1.26 (1.26), 1.07 (1.075), 0.893 (0.8916), 0.817 (0.8182), 0.73 (0.728), 0.68 (0.686) Å. Elemental analysis by analytical electron microscopy showed a small amount of Cl (contamination or surface-bound Cl from HCl?) as well as Cr and Fe (submicrometre-sized ferri-chromite⁷). Examination by high-resolution mass spectrometry at 70–550 °C showed only small amounts of volatile material, probably contamination. Peaks appeared up to at least $m/e = 733$, but none of the more abundant ones corresponded to C₆₀ or other C_n ions.

To check whether such diamonds are present in other meteorites containing Xe-HL, we isolated similar residues from Murchison (C2), Murray (C2) and Indarch (EH4), using HClO₄ at higher temperature (190–206 °C) to remove the more reactive forms of carbon. Residues Murchison GL1 (white, 225 p.p.m.) and Indarch CF (dark grey, 36 p.p.m.) both showed diamond by electron diffraction. Murray A2C (white, 250 p.p.m.) was not checked by electron diffraction but showed only minor Al and Si on the SEM.

From the TEM work it appears that some amorphous, low-atomic-number material is present in addition to diamond, perhaps radiation-damaged diamond or unrelated extraneous material. (As we note later, Xe-HL seems to have been trapped by ion implantation, and presumably was accompanied by large amounts of other ions.) This is supported by density-gradient



Fig. 1 Dark-field transmission electron micrograph of Allende CJ, in which bright areas are diamond grains diffracting electrons into one part of the innermost ring of the diffraction pattern shown in Fig. 2. As most grains do not have the proper orientation, the true abundance of diamond is perhaps five times higher than indicated by the bright areas in the picture.

centrifugation, showing that Murchison GL1 has an effective bulk density of 2.22–2.33 g cm⁻³, well below the density of pure diamond (3.51 g cm⁻³). This material is not graphite or amorphous carbon, else the sample would not be pure white. But its amount must be less than implied by the low effective density, as the particles sink not singly but as aggregates, containing much interstitial liquid immobilized in angstrom-sized voids.

Curiously the infrared spectrum strongly resembles that of soot, with peaks at 2.93, 3.17, 5.59, 6.13 and 7.14 μm . For soot, these features are attributed to the reaction of surface atoms with H and O, and presumably such surface alteration also occurs for fine-grained diamond. Approximately 9% of the atoms in 50-Å diamond are at the surface.

On heating, C δ showed a surface graphitization behaviour consistent with that of diamond. A sample wrapped in platinum foil did not change when heated in air (19 days at 165 °C, 3 days at 280 °C, 1 day at 380 °C), but blackened visibly at $\sim 10^{-2}$ torr (1 day at 380 °C). A sample heated in a Pyrex tube in high vacuum darkened only slightly (1 h at 100–550 °C, 3 min at 550 °C). Diamond behaves similarly, darkening at low O₂ pressures but not in high vacuum or at high O₂ pressures (where any graphite formed is rapidly oxidized).

Given that C δ is mainly diamond, is the diamond the actual carrier of the isotope anomalies? We cannot get absolute proof, as our samples are not pure diamond; the best we can do is establish correlations between the anomalies and the diamond content of the samples.

The Xe-HL content is indicated by ^{136}Xe -HL (Table 1). It is generally highest in the white samples, although black Allende B1B is close and white Murray A2C (which contained some extraneous phases) is lower. Apparently Xe-HL stays with the diamond when other phases are destroyed. The highest values for Allende and Murchison are similar ($17\text{--}19 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$), as expected if both meteorites contained the same kind of C δ .

The correlation with other anomalies is more tenuous, as no data are available yet on the new samples. Earlier measurements by stepped combustion show that light N, light C, and Xe-HL are all released in the same temperature range of 500–600 °C^{1,3,8}, and hence may be located in the same phase. However, this correlation must be checked on diamond-enriched samples. (The combustion temperature of terrestrial diamond under identical conditions is ~ 250 °C higher⁹, but that is a trivial consequence of the coarser grain size. At an activation energy of 60 kcal mol⁻¹, the combustion rate per unit area is 4.8×10^3 times higher at 800 °C than at 550 °C, but this difference would be exactly offset

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by a 70-fold difference in grain size, that is, 3,500 Å rather than 50 Å).

Diamonds have been previously found in some meteorites (most ureilites and two iron meteorites), but they apparently formed by shock, in terrestrial or asteroidal impacts^{10,11}. It seems unlikely that the Cδ diamonds originated in this way. First, although all four meteorites in which these diamonds have been found are somewhat brecciated (Allende least so), none shows the strong shock effects, high post-shock temperatures, or both, that characterize other diamond-bearing meteorites. Second, all the Cδ-bearing meteorites also contain 10³–10⁴ p.p.m. of graphitic or amorphous carbon that has no Xe-HL or light N and is isotopically heavier ($\delta^{13}\text{C} = -15\%$, compared with -38% for Cδ^{1,12}). If the diamonds were made from graphite grains in the meteorite parent bodies by shock, why did the shock (in three or four different parent bodies) single-mindedly transform only the Xe-HL-bearing grains, while ignoring the more abundant, gas-poor carbon grains? Third, all four meteorites have essentially similar Cδ, with comparable gas contents and isotopic compositions (Table 1). If the diamonds were shock-produced, we would expect greater variations, depending on the proportions of the two types of carbon converted to diamond and on post-shock diffusion losses. Ureilites, for example, show a ~100-fold variation in gas concentration¹³.

By default, it seems necessary to invoke an extra-solar origin for the diamond. But diamond has not been seriously considered a possible constituent of the cosmos outside the Solar System, as no astronomical object except planets has the right pressure-temperature conditions for its formation as a stable phase (40–400 kbar, 1,200–4,000 K), and any diamonds formed in planets could not get out because of the high escape velocity. (Landau¹⁴ has pointed out that diamond of a steep size distribution would give a good match to the interstellar extinction curve, but he did not consider the origin of the diamonds.) Although shocks are common in the cosmos, they too, do not give the combination of high pressure and moderate temperature required for formation of diamond. The only feasible mechanism would seem to be metastable formation of diamond from a hot gas at low pressures, as recently demonstrated in the laboratory¹⁵. Let us see how this mechanism can be incorporated in a scheme that accounts for Xe-HL.

Xe-HL apparently formed in a supernova¹⁶ and (along with other noble gases, H and N) was trapped in carbon grains by ion implantation¹⁷. The high relative velocities (~10³ km s⁻¹) required for the latter process imply that the grains predate the supernova, and presumably formed in the gas shell ejected by the pre-supernova during its red giant or planetary nebula stage some 10²–10⁴ yr earlier¹⁸. Carbon is the first solid expected to

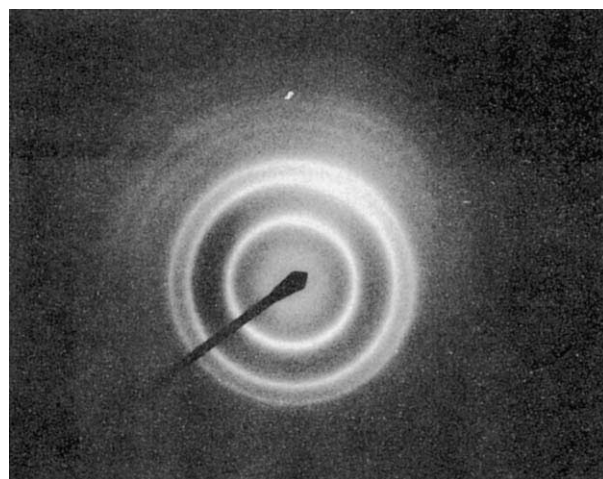


Fig. 2 Selected-area electron diffraction pattern from Allende CJ. This is a typical ring powder pattern, with *d*-values matching those of diamond.

condense from a carbon-rich gas at equilibrium¹⁹, and although graphite is the stable phase, diamond, being metastable by only ~2 kJ mol⁻¹, could form instead. Indeed, such preferential formation of diamond has been observed in the laboratory¹⁵. The processes studied so far involve decomposition of CH₄ or organic compounds in the presence of large amounts of hydrogen, either by ordinary pyrolysis or by plasma reactions. The mechanism is not yet known, but seems to involve CH₃ radicals and H atoms. Although CH₃ is not an abundant radical in red giants, other radicals or molecules, such as CH, C₂H and C₂H₂, may be able to serve in its place. Whatever the mechanism, it now seems necessary to add diamond to the list of interstellar grain constituents. We review here some of the prospects opened up by this work.

First, diamond should be looked for in space wherever graphite is thought to be present: circumstellar, interstellar and interplanetary dust (including dust from comets). Perhaps the abundance of interstellar graphite has been overestimated; Nuth²⁰ has pointed out that most of the carbon in primitive meteorites is a complex organic polymer, not graphite, and now that Cδ has turned out to be diamond, the balance is even more lopsided: graphite constitutes ≤2,000 p.p.m., compared to 100–400 p.p.m. diamond and (2–3) × 10⁴ p.p.m. organic carbon. But meteorites may not be representative of interstellar matter. Several authors have argued that a single, massive supernova exploded near the forming Solar System, injecting short-lived

Table 1 Anomalous Xe in diamond-rich meteorite separates

Sample		Class	Mass fraction* p.p.m.	Gas content 10 ⁻⁸ cm ² g ⁻¹		$\frac{^{136}\text{Xe}}{^{132}\text{Xe}}$	References
				¹³² Xe	¹³⁶ Xe-HL†		
Allende	CJ‡	C3V	89	30±6	19±4	0.642±3	This paper
Allende	CH‡	C3V	109	24±2	15±1	0.649±3	This paper
Allende	B1B	C3V	390	25±3	16±2	0.644±5	3
Allende	CC	CeV	180	21±2	14±1	0.653±23	3
Allende	B1E	C3V	180	16±2	10±1	0.636±8	This paper
Allende	CG	C3V	250	12±1	8±1	0.652±3	6
Murchison	GL1‡	C2	225	37±7§	17±3	0.550±2§	This paper
Murchison	2C10f	C2	780	18±2§	8±1	0.537±2§	23
Murray	A2C‡	C2	250	28±2§	12±1	0.535±2§	This paper
Indarch	CF	EH4	36	9±2	5±1	0.593±4	This paper

* Mass fraction of separate relative to bulk meteorite. Values for different samples are not directly comparable. In the earlier samples they are high due to extraneous phases and in the later, highly purified, samples they are low due to mechanical losses.

† ¹³⁶Xe-HL is the calculated excess ¹³⁶Xe in the sample, relative to planetary Xe with ¹³⁶Xe/¹³²Xe = 0.32.

‡ White; all other samples are black (except Indarch CF, which is dark grey) owing to the presence of graphitic carbon.

§ Excluding an isotopically normal Xe component released at 800 °C, before the release of Xe-HL (≥1,000 °C). It occurs only in C2 chondrites²³. Its carrier, C₂ is carbonaceous but totally uncharacterized.

radionuclides as well as excess amounts of the stable isotopes ^{12}C , ^{16}O and ^{20}Ne (refs 21, 22). If the Solar System is indeed enriched in supernova products, then C δ and Xe-HL (whether from the same or another supernova) may be atypically high.

Second, if diamond is detected in circumstellar dust, its formation conditions (as inferred from the atmosphere of the central star) should be determined and duplicated, insofar as possible, in the laboratory. Possibly nature makes diamond more efficiently than even the best laboratory synthesis discovered so far.

Finally, meteoritic diamond should be isotopically analysed for all detectable elements. If the xenon was trapped by ion implantation from a supernova, then it ought to be accompanied by the remainder of the periodic table. Diamond, owing to its extreme durability, may be an informative record of stellar nucleosynthesis and chemistry and of the prehistory of the Solar System.

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- Swart, P. K., Grady, M. M., Pillinger, C. T., Lewis, R. S. & Anders, E. *Science* **220**, 406–410 (1983).
- Lewis, R. S., Srinivasan, B. & Anders, E. *Science* **190**, 1251–1262 (1975).
- Lewis, R. S., Anders, E., Wright, I. P., Norris, S. J. & Pillinger, C. T. *Nature* **305**, 767–771 (1983).
- Lewis, R. S., Tang, M., Wacker, J. F. & Steel, E. *Lunar planet. Sci.* **18**, 550–551 (1987).
- Heymann, D. *J. geophys. Res.* **91**, E135–E138 (1986).
- Lewis, R. S., Anders, E., Shimamura, T. & Lugmair, G. W. *Science* **222**, 1013–1015 (1983).
- Fraundorf, P., Flynn, G. J., Shirek, J. R. & Walker, R. M. *Earth planet. Sci. Lett.* **37**, 285–295 (1977).
- Frick, U. & Pepin, R. O. *Earth planet. Sci. Lett.* **56**, 64–81 (1981).
- Swart, P. K., Grady, M. M., Wright, I. P. & Pillinger, C. T. *J. geophys. Res.* **87**, 283–288 (1982).
- Lipschutz, M. E. & Anders, E. *Geochim. cosmochim. Acta* **24**, 83–105 (1961).
- Clarke, R. S. Jr, Appleman, D. E. & Ross, D. R. *Nature* **291**, 396–398 (1981).
- Ott, U., Kronenbitter, J., Flores, J. & Chang, S. *Geochim. cosmochim. Acta* **48**, 267–280 (1984).
- Göbel, R., Ott, U. & Begemann, F. *J. geophys. Res.* **83**, 855–867 (1978).
- Landau, R. *Nature* **226**, 924 (1970).
- Roy, R. *Nature* **325**, 17–18 (1987).
- Heymann, D. & Dziczkaniec, M. *Proc. Lunar planet. Sci. Conf.* **10**, 1943–1959 (1979).
- Lewis, R. S. & Anders, E. *Astrophys. J.* **247**, 1122–1124 (1981).
- Clayton, D. D. *Proc. Lunar planet. Sci. Conf.* **128**, 1781–1802 (1981).
- Larimer, J. W. & Bartholomay, M. *Geochim. cosmochim. Acta* **43**, 1455–1466 (1979).
- Nuth, J. A. *Nature* **318**, 166–168 (1985).
- Cameron, A. G. W. & Truran, J. W. *Icarus* **30**, 447–461 (1977).
- Schramm, D. N. & Olive, K. A. *Ann. N.Y. Acad. Sci.* **395**, 236–241 (1982).
- Alaerts, L., Lewis, R. S., Matsuda, J. & Anders, E. *Geochim. cosmochim. Acta* **44**, 189–209 (1980).

Metastable carbon in two chondritic porous interplanetary dust particles

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An understanding of carbonaceous matter in primitive extraterrestrial materials is an essential component of studies on dust evolution in the interstellar medium and the early history of the Solar System. We have suggested previously that a record of graphitization is preserved in chondritic porous (CP) aggregates and carbonaceous chondrites^{1,2} and that the detailed mineralogy of CP aggregates can place boundary conditions on the nature of both physical and chemical processes which occurred at the time of their formation^{2,3}. Here, we report further analytical electron microscope (AEM) studies on carbonaceous material in two CP aggregates which suggest that a record of hydrocarbon carbonization may also be preserved in these materials. This suggestion is based upon the presence of well-ordered carbon-2H (lonsdaleite) in CP aggregates W7029*A and W7010*A2. This carbon is a metastable phase resulting from hydrous pyrolysis below 300–350 °C and may be a precursor to poorly graphitized carbons (PGCs) in primitive extraterrestrial materials².

Chondritic porous aggregates are samples of unmelted micrometeorites that survived atmospheric entry without

appreciable effects of thermal metamorphism due to pulse-heating^{3–5}. Broadly based classification studies and chemical analyses of stratospheric dust collections show that fluffy aggregates of small-sized grains (<100 nm) with a chondritic bulk composition are commonly of extraterrestrial origin^{6,7}. The bulk carbon content of CP aggregates can range up to 5 wt% in carbon-rich CP aggregates⁶ and possibly includes hydrocarbon compounds⁸. For this study we selected two CP aggregates from the Johnson Space Center (JSC) Cosmic Dust Collection. Detailed information on collection, curation and processing procedures at the JSC Curatorial Facility are given by Rietmeijer and Mackinnon³ and references therein.

Chondritic porous aggregate W7029*A is a carbon-rich, uncontaminated sample of primitive extraterrestrial material³. During preparation for AEM study parts of this aggregate broke apart on to the holey carbon substrate. Thus, individual particles from the aggregate were suitably dispersed and accessible to study^{2–4}. In CP aggregate W7029*A about 44% of all individual particles are carbonaceous material which occurs as (1) poorly graphitized carbon²; (2) amorphous carbon⁹ and (3) a well-ordered carbon phase. The remainder of grains in this CP aggregate are predominantly low-temperature minerals³. The presence of kaolinite³ and cubic Bi₂O₃⁴ in this CP aggregate suggests that temperatures on atmospheric entry were no higher than ~300 °C. While CP aggregate W7010*A2 also belongs to the carbon-rich group of CP aggregates, its carbonaceous material mainly occurs in its matrix as the embedding medium for ultra fine-grained (1–150 nm) silicates, sulphides and oxides¹⁰. Only a small portion of individual grains in this aggregate is PGC and a well-ordered carbon phase.

Analyses were performed with a modified JEOL 100CX AEM⁹ equipped with a Princeton Gamma Tech (PGT) System IV Energy Dispersive Spectrometer (EDS) operating at an accelerating voltage of 100 kV. All EDS spectra are consistent with the presence of low atomic number ($Z < 11$) elements only. For selected grains we confirmed the carbon chemistry using a JSM-35CF scanning electron microscope fitted with a PGT Windowless EDS (WEDS) operating at an accelerating voltage of 10 kV and by WEDS and electron energy loss spectroscopy (EELS) using a Philips EM 420T operating at 120 kV. The samples are not provided with a conductive surface coating (for example, Au/Pd or C) before analysis. Sample contamination with carbon materials during laboratory handling or in the AEM is negligible^{3,11}. Identification of phases is possible through a combination of AEM imaging, selected area electron diffraction (SAED), EDS, WEDS and EELS analyses. The diffraction spacings shown in Table 1 have a relative error of ~1%. For individual particles we obtained SAED data at various sample tilt angles relative to the incident electron beam.

The majority of carbonaceous material in CP aggregate W7029*A is present as clumped masses of thin sheets and as irregular fluffy grains. The morphology and crystallographic properties of material in these occurrences are consistent with poorly graphitized carbon². Thin, amorphous carbon rims on pre-existing minerals similar to those observed in other CP aggregates¹² are also present in CP aggregate W7029*A⁴. In addition, a small fraction (~10%) of carbonaceous material in this CP aggregate occurs as thin (<100 Å) electron transparent sheets which range from 0.2 µm × 0.3 µm to 0.6 µm × 2.0 µm in size (Figs 1 and 2). In general, these sheets have lobate circumferences, but in rare cases show a subhedral outline. Most of this carbonaceous material occurs as single sheets, although stacking of sheets is occasionally observed. When exposed to the electron beam the smooth surfaces of these sheets may become slightly wrinkled, suggesting loss of volatiles. In CP aggregate W7010*A2, the morphology of carbonaceous grains which are not part of the matrix, is similar to the thin sheets in CP aggregate W7029*A. SAED data for each aggregate shown in Table 1 are for the carbon phase in these sheets and are consistent with crystalline carbon-2H, or lonsdaleite. However, the orientation

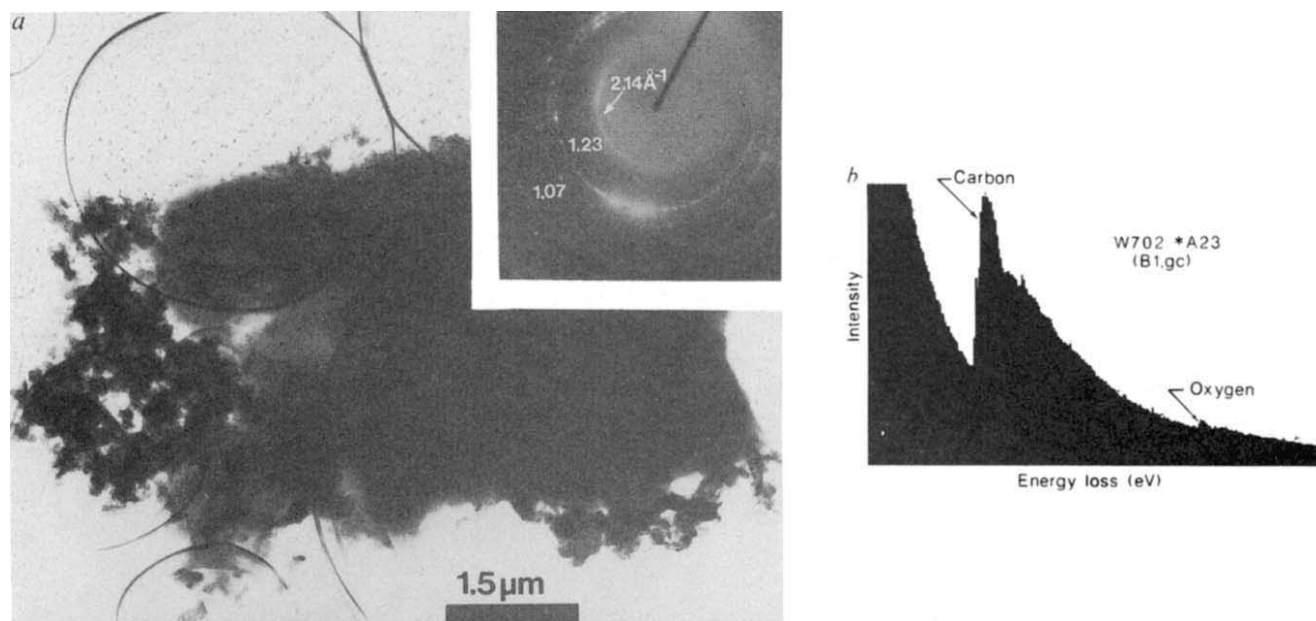


Fig. 1 *a*, Large carbon-2H with regions of poorly graphitized carbon (left-hand side). The SAED pattern (inset) is consistent with carbon-2H. *b*, Electron energy loss spectrum on thin, smooth carbon-2H grain shown in *a*.

of the thin sheets parallel to the sample support film interferes with acquisition of all diffraction spacings typical of carbon-2H. With the exception of two diffraction spacings, all other diffraction spacings for carbon-2H and PGC in the samples overlap. Nevertheless, in conjunction with the known absence of high-*Z* elements in these sheets and the fact that no known carbon phase mimics these two diffraction spacings¹³, we identify the thin sheets as crystalline carbon-2H.

The SAED data (Table 1) show that carbons in CP aggregate W7029*A, and to a lesser extent in CP aggregate W7010*A2, occur mainly in two distinct forms as carbon-2H and PGC. In the PGCs the d_{002} basal spacing is typically at 3.50 ± 0.03 Å. However, the majority of PGC particles in both CP aggregates also show carbon-2H interplanar spacings and this suggests that these carbon particles are a mixture of PGC and carbon-2H. For comparison, interplanar spacings for carbon residues from

carbonaceous chondrites are also listed in Table 1. Carbons in these meteorites are primarily PGC¹⁴.

We have previously suggested that PGC in CP aggregate W7029*A and in carbonaceous chondrites results from graphitization of a carbon phase². The graphite reflection, d_{101} (2.10 Å) in PGC from CP aggregates W7029*A and W7010*A2 is more diffuse and less defined when compared to the same reflection from PGC in carbonaceous chondrites. This reflection is in fact a composite of the (101) and (100) reflections ($d = 2.03$ Å and 2.13 Å) and is a sensitive indicator of the onset of graphitization¹⁵. The value of the (002) spacing in PGC is a measure of the degree of order, or the extent of graphitization, for PGC^{2,15}. The above observations suggest that graphitization in carbonaceous chondrite PGC is better developed than that observed for PGC in CP aggregates.

In order to understand the occurrence of carbon-2H in CP

Table 1 Interplanar spacings (Å) for carbon sheets from 11 and 9 individual grains in CP aggregates W7029*A and W7010*A2, respectively, compared with interplanar spacings (Å) for carbon-2H and poorly graphitized carbons in CP aggregate W7029*A and selected carbonaceous chondrites

<i>hkl</i>	W7029*A	W7010*A2	Carbon-2H	<i>hkl</i>	W7029*A	Allende CV3	Cold Bokkeveld C2	Orgueil CI
10 $\bar{1}$ 0	2.185 ± 0.015	2.17	2.18	002	3.50 (3.47–3.53)	3.47 (3.39–3.51)	3.6 (3.4–3.9)	3.8 (3.5–4.1)
0002	—	—	2.06	100	2.18 ± 0.03	—	—	—
10 $\bar{1}$ 1	—	—	1.93		2.09	2.10	2.10	2.09
				004	1.75 ± 0.03	1.73	—	—
10 $\bar{1}$ 2	—	—	1.50		1.635	—	—	—
11 $\bar{2}$ 0	1.26 ± 0.01	1.265	1.26	—	1.265 ± 0.015	—	—	—
	1.195 ± 0.01	1.19		110	1.19 ± 0.02	1.22	1.21	1.21
10 $\bar{1}$ 3	—	—	1.17	006	1.16	1.16	—	—
11 $\bar{2}$ 2	1.085 ± 0.015	1.09	1.075	200	1.09 ± 0.02	1.06	1.05	—
				106	1.01	—	—	—
					0.92	—	—	—
					0.87	—	—	—
22 $\bar{1}$ 0	0.81 ± 0.02	—	—	210	0.82 ± 0.02	0.83	—	—
	0.72 ± 0.015	—	—	300	0.725 ± 0.015	0.71	—	—

Data for carbon 2H from ref. 19, for W7029*A from ref. 2 and for carbonaceous chondrites from ref. 14.

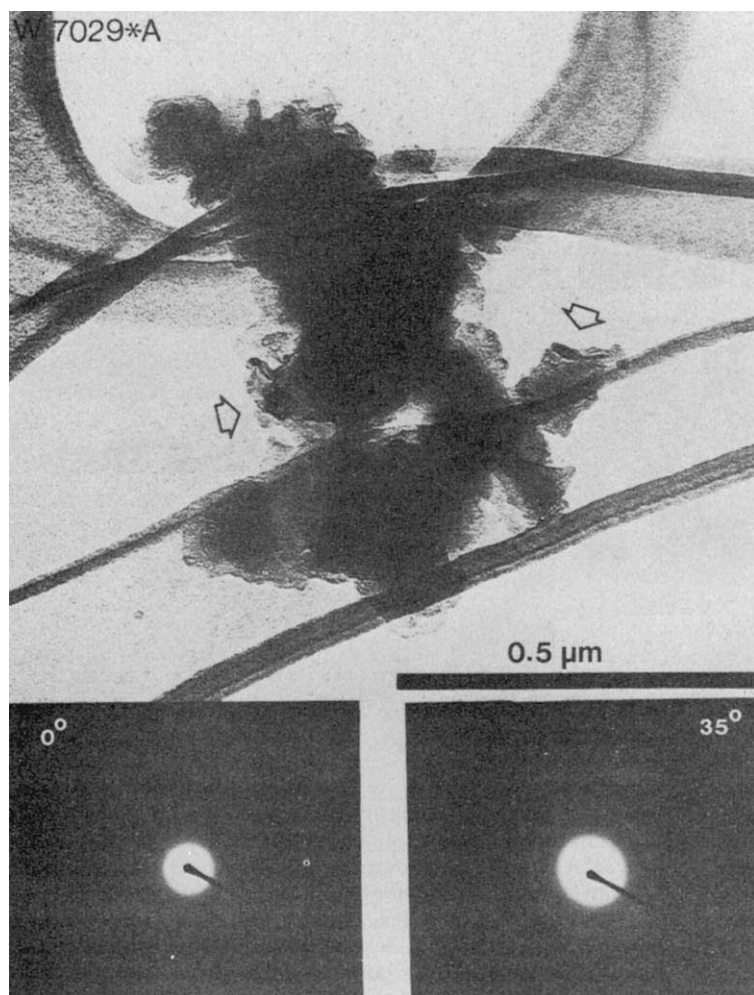


Fig. 2 Carbon-2H in CP aggregate W7029*A showing small areas of electron beam damage (arrowed). SAED patterns are shown for two different tilt orientations relative to the incident electron beam and are typical for carbon-2H.

aggregates, we refer to literature on low-temperature alterations of (hydro-) carbon compounds because the presence of layer silicates and PGC in these CP aggregates indicate aqueous activity at temperatures $<315^{\circ}\text{C}$ ^{2,3}. As a likely reaction path may be hydrous pyrolysis of hydrocarbons, we would like to draw attention to materials and conditions prevalent during hydrous pyrolysis of kerogens in natural terrestrial environments^{16,17} and to those that are represented in CP aggregate mineralogy^{2,3}. In addition, we discuss the formation of carbon-2H as a low-temperature metastable phase because other processes for the formation of stable carbon-2H in an interstellar, or early Solar System, environment are not readily invoked for primitive extraterrestrial materials such as CP aggregates. For example, carbon-2H is a stable high-temperature and high-pressure polymorph of carbon that can form from pure graphite at temperatures $>1,000^{\circ}\text{C}$ and static pressures $>130\text{ kbar}$ ¹⁸. Carbon-2H may also form via intense shock metamorphism of pure graphite^{19,20}. However, solar flare track⁵ and noble gas²¹ data show that CP aggregates are not part of larger (for example, meteoroid size) bodies during Solar System transit. Thus, intense shock events through impact with meteoroids, or larger-sized bodies, are unlikely formation mechanisms for carbon-2H in CP aggregates. In addition, parent bodies for CP aggregates are probably porous and of low density (for example, comet-like²²) and would not provide an environment suitable for high-temperature and/or high-pressure transformations. It is conceivable that carbon-2H resulting from a parent body brecciation process may have been introduced as a foreign component to the CP aggregates. Two lines of evidence argue against introduc-

tion of carbon-2H that formed elsewhere prior to CP aggregate formation: (1) lack of evidence for shock metamorphism in silicates (for example, olivines) in the CP aggregates²³ which would be expected from a brecciation process²⁴ and (2) the association of carbon-2H and PGC in CP aggregate W7029*A. Thus, we explore carbon-2H formation by a process other than shock metamorphism, such as *in situ* formation by hydrous pyrolysis³. This process in terrestrial environments produces metastable carbon-2H²⁵.

Catalytically activated hydrous pyrolysis of hydrocarbons, particularly kerogens, is an important process for the diagenesis of terrestrial hydrocarbon deposits at temperatures $<350^{\circ}\text{C}$ ^{16,17,26}. Kerogen is thermodynamically unstable and in a terrestrial environment, with increasing time and temperature, will decompose into successively more stable phases such as graphite, CO_2 , CH_4 , and N_2 . This irreversible process may proceed along complex pathways with a variety of intermediate phases^{17,26,27}. Also, the pyrolysis temperature of the reactions is more important than the effect of total pressure or the presence of a catalyst^{17,26}. An important fraction of carbonaceous material in micrometeorites may occur as hydrocarbons as suggested by infrared and ultraviolet spectra of circum- and interstellar dust, the physical properties of carbons in CP aggregates and optical properties of carbonaceous material in several CP aggregates^{8,23}. As we have shown in previous studies on PGC and layer silicates in CP aggregate W7029*A, the physical environment (for example, pressure and temperature conditions) which characterizes the aggregate compares favourably with terrestrial hydrous pyrolysis^{2-4,22}. In addition, layer silicates similar to that which

catalytically activate terrestrial hydrous pyrolysis¹⁶ have been observed in CP aggregates^{3,28}. The efficiency of the catalytic process depends, among other parameters, on the possibility of readsorption of pyrolysis products into the surface of catalysts¹⁶. In this respect, the porous structure of the aggregates may be conducive to exposure of large amounts of surface area to an active aqueous fluid^{3,22}. Most importantly, of all possible pyrolysis processes, only hydrous pyrolysis produces the soft, that is well-graphitizable, carbons²⁶ which, with continued heat-treatment, can transform to PGC¹⁵. We propose that at the low temperatures required for these primitive parent bodies²², the kinetics of alteration reactions will dominate this catalytic hydrous pyrolysis mechanism. Thus, for conditions likely to occur after CP aggregate formation (see above) carbon-2H forms as a crystalline metastable product of catalytically activated hydrous pyrolysis of hydrocarbons (following the Ostwald step rule) in primitive extraterrestrial materials. This proposition may even prove tenable for some carbons in many carbonaceous chondrites^{29,30}. For example, a variety of layer silicates have been observed in CP aggregates^{3,23} and in CI and CM carbonaceous chondrites³¹. Previous observations on carbon phases in both types of primitive extraterrestrial materials² as well as the possible conditions of their formation^{1,22,29} suggest that low-temperature diagenesis involving catalytic reactions may be a primary mechanism for the formation of the mineralogy observed in many CP aggregates and some components of carbonaceous chondrites. A precise understanding of the complex pathways for the formation of carbonaceous compounds and carbon phases may allow detailed interpretations of isotopic variations (for example changes in D/H ratios, see ref. 27) noted for CP aggregates³² and carbonaceous chondrites³³.

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- Rietmeijer, F. J. M. & Mackinnon, I. D. R. *Lunar planet. Sci.* **16**, 700-701 (1986).
- Rietmeijer, F. J. M. & Mackinnon, I. D. R. *Nature* **316**, 733-736 (1985).
- Rietmeijer, F. J. M. & Mackinnon, I. D. R. *J. geophys. Res. suppl.* **90**, D149-D155 (1985).
- Mackinnon, I. D. R. & Rietmeijer, F. J. M. *Nature* **311**, 135-138 (1984).
- Bradley, J. P., Brownlee, D. E. & Fraundorf, P. *Science* **226**, 1432-1434 (1984).
- Brownlee, D. E., Rajan, R. S. & Tomandl, D. A. in *Comets, Asteroids, Meteorites interrelations, evolution and origins* (ed. Delsemme, A. H.) 137-150 (University of Toledo, 1977).
- Mackinnon, I. D. R., McKay, D. S., Nace, G. A. & Isaacs, A. M. *J. geophys. Res. suppl.* **87**, A413-A421 (1982).
- Rietmeijer, F. J. M. in *Interrelationships among Circumstellar, Interstellar and Interplanetary Dust* (eds Nuth, J. A. III & Stencil, R. E.) A23-A27 (NASA Conf. Publ. 2403, 1985).
- Mackinnon, I. D. R., Rietmeijer, F. J. M., McKay, D. S. & Zolensky, M. E. in *Microbeam Analysis—1985* (ed. Armstrong, J. T.) 291-297 (San Francisco Press, 1985).
- Rietmeijer, F. J. M. & McKay, D. S. *Lunar planet. Sci.* **17**, 710-711 (1986).
- Rietmeijer, F. J. M. *Meteoritics* **20**, 43-48 (1985).
- Bradley, J. P., Brownlee, D. E. & Fraundorf, P. *Science* **223**, 56-58 (1984).
- Derjaguin, B. V., Fedoseev, D. V., Varnin, V. P. & Vnukov, S. P. *Nature* **269**, 298-299 (1977).
- Lumpkin, G. R. *Lunar planet. Sci.* **14**, 450-451 (1983); in *Lunar. planet. Sci.* **17**, 502-503 (1986).
- Jenkins, G. M. & Kawamura, K. *Polymeric Carbons—Carbon Fibre, Glass and Char* (Cambridge University Press, 1976).
- Galwey, A. K. *Nature* **223**, 1257-1260 (1969); *Geochim. cosmochim. Acta* **36**, 1115-1130 (1972).
- Long, G., Neglia, S. & Favretto, L. *Geochim. cosmochim. Acta* **32**, 647-656 (1968).
- Bundy, F. P. & Kasper, J. S. *J. chem. Phys.* **46**, 3437-3446 (1967).
- Frondel, C. & Marvin, U. B. *Nature* **214**, 587 (1967).
- Hanneman, R. E., Strong, H. M. & Bundy, F. P. *Science* **155**, 995-997 (1967).
- Hudson, B., Flynn, G. J., Fraundorf, P., Hohenberg, C. M. & Shirck, J. *Science* **211**, 383-386 (1981).
- Rietmeijer, F. J. M. *Nature* **313**, 293-294 (1985).
- Mackinnon, I. D. R. & Rietmeijer, F. J. M. *Rev. Geophys.* (in the press).
- Dodd, R. T. *Meteorites* (Cambridge University Press, 1981).
- Jedwab, J. & Boulegue, J. *Nature* **310**, 41-43 (1984).

- Fitzer, E., Mueller, K. & Schaeffer, W. in *Chemistry and Physics of Carbon*. Vol. 7 (ed. Walker, P. L. Jr) 238-383 (Dekker Inc, New York, 1971).
- Hoering, T. C. *Yb. Carnegie Instn Wash.* **81**, 397-402 (1982); *Yb. Carnegie Instn Wash.* **82**, 386-390 (1983); *Org. Geochem.* **5**, 267-278 (1984).
- Tomeoka, K. & Buseck, P. R. *Earth planet. Sci. Lett.* **69**, 243-254 (1984).
- Rietmeijer, F. J. M. *Lunar planet. Sci.* **16**, 698-699 (1985).
- Anders, E., Hayatsu, R. & Studier, M. H. *Science* **182**, 781-790 (1973).
- Barber, D. J. *Clay Miner.* **20**, 415-454 (1985).
- McKeegan, K. D., Walker, R. M. & Zinner, E. *Geochim. cosmochim. Acta* **49**, 1971-1987 (1985).
- Kerridge, J. F. *Earth planet. Sci. Lett.* **64**, 186-200 (1983).

A novel observational test of momentum balance in a solar flare

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A unique combination of Solar Maximum Mission X-ray spectra and Sacramento Peak Observatory H α imaging spectra has enabled us, for the first time, to measure and compare momentum values of upflowing and downflowing plasmas during the impulsive phase of a solar flare. We observed the well-known blue asymmetry of X-ray spectral lines¹, indicative of upflow, in the coronal Ca XIX line. We simultaneously observed the red asymmetry of H α line profiles, indicative of downflow, in bright H α kernels. We find that, to within observational uncertainty, the momentum transported by the upflowing X-ray plasma was the same as that of the downflowing H α material. Of the several physical mechanisms advanced² to explain the observed blue asymmetry of X-ray lines, only explosive chromospheric evaporation predicts oppositely directed momenta of equal amplitude.

Impulsive hard X-rays from the small C2 flare were detected by the Solar Maximum Mission (SMM) hard X-ray burst spectrometer from 23:43:42 to 23:44:33 on 30 April 1985. Before this burst the region showed enhanced counts in the O VIII and Mg XI channels of the SMM flat crystal spectrometer (FCS). From the flux ratios we infer a preflare coronal temperature $T_0 \approx 3 \times 10^6$ K. According to coronal loop equilibrium scaling laws^{3,4} and the loop length inferred from the H α flare images, this corresponds to a preflare pressure of $P_0 \approx 12$ dyn cm⁻² in the corona and upper chromosphere.

Soon after the peak of soft X-ray emission, at 23:45 UT the FCS was pointed to the brightest feature within its field and a spectroscopic scan of the helium-like Ne IX triplet was obtained. Following the technique of Wolfson *et al.*⁵ we derive a coronal electron density of $n_e \approx 8 \times 10^{10}$ cm⁻³. The SMM bent crystal spectrometer (BCS) observed the whole flare, both spatially and temporally, but to measure accurately the Doppler shift of the blue component of the Ca XIX spectrum it was necessary to average over the 70 s time period preceding soft X-ray maximum. Figure 1 compares these data to synthetic spectra. From this fit we derive a mean value of the upflow velocity $v_e \approx 290 \pm 100$ km s⁻¹. From the strength of the Ca XIX line as a whole we derive a total coronal emission measure $EM \approx 3 \times 10^{48}$ cm⁻³ at soft X-ray maximum.

H α imaging spectra⁶ were obtained using the vacuum tower telescope at the Sacramento Peak Observatory (SPO). Preflare-subtracted H α line profiles in the brightest H α -emitting pixel at representative times during the impulsive hard X-ray burst

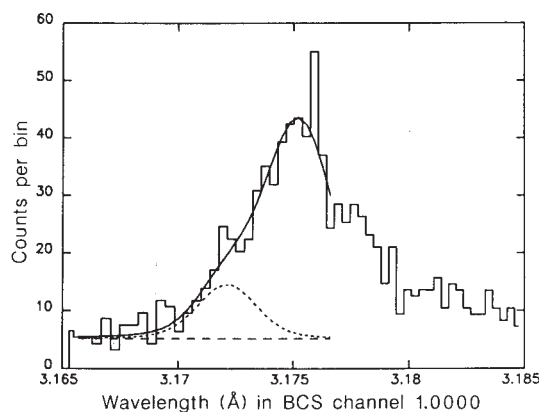


Fig. 1 A comparison of Ca XIX spectra observed with the SMM bent crystal spectrometer and a synthetic spectral fit. The continuous curve is the synthetic fit, which includes the background indicated by the long-dashed line, a symmetric unshifted component, and a blue-shifted component given by the short-dashed curve, implying an upflow velocity of $290 \pm 100 \text{ km s}^{-1}$.

are shown in Fig. 2. For the ensuing analysis we have examined all pixels that contribute to the total redshifted region of cross-sectional area $S_c \approx 4 \times 10^{17} \text{ cm}^2$. On average, profiles in this region show a peak redshift velocity of $\sim 60 \text{ km s}^{-1}$, which falls by a factor of 2 in about 35 s. For purposes of comparison with the X-ray data we will adopt an average velocity $v_e \approx 30 \text{ km s}^{-1}$ over a characteristic time $\tau_e \approx 70 \text{ s}$.

Following the method of Ichimoto and Kurokawa⁷, we derive the momentum of the coronal plasma by assuming that the total emission measure $\text{EM} = n_e^2 V$ of the soft X-ray emitting plasma of density n_e and volume V is provided by chromospheric evaporation in a timescale τ_e . Thus, conservation of mass yields the relation

$$mn'v_e S_c \tau_e = mn_e V = \frac{m \text{EM}}{n_e} \quad (1)$$

where m , n' , v_e and S_c are the mean mass per nucleon, density, velocity, and cross-sectional area of upflowing plasma, and we do not assume that $n' = n_e$. The total momentum that is associated with this material is

$$p_e = mn'v_e^2 S_c \tau_e \quad (2)$$

where we have assumed constant v_e during τ_e . By eliminating n' , S_c , and τ_e we obtain

$$p_e = \frac{mv_e \text{EM}}{n_e} \quad (3)$$

Substituting parameter values from the previous section, we obtain $p_e \approx 2 \times 10^{21} \text{ g cm s}^{-1}$.

Explosive chromospheric evaporation⁸ creates a cool downward-moving condensation⁹ behind a radiating shock¹⁰. Its rate of mass accretion per unit area is given by $mn_0 v_c$, where n_0 is the preflare (unshocked) chromospheric density and v_c is the downward condensation velocity. Consequently its momentum after τ_c is approximately

$$p_c = mn_0 v_c^2 S_c \tau_c \quad (4)$$

Assuming chromospheric-coronal pressure equilibrium before the flare, the inferred preflare pressure $P_0 \approx 12 \text{ dyn cm}^{-2}$ and a typical preflare chromospheric temperature $T_0 \approx 8,000 \text{ K}$ imply a preflare density $n_0 \approx 1 \times 10^{13} \text{ cm}^{-3}$. We therefore estimate that $p_c \approx 6 \times 10^{21} \text{ g cm s}^{-1}$.

The almost equal amplitudes and opposite signs of the momenta of upflowing X-ray and downflowing H α material have interesting implications for the debate² over the physical

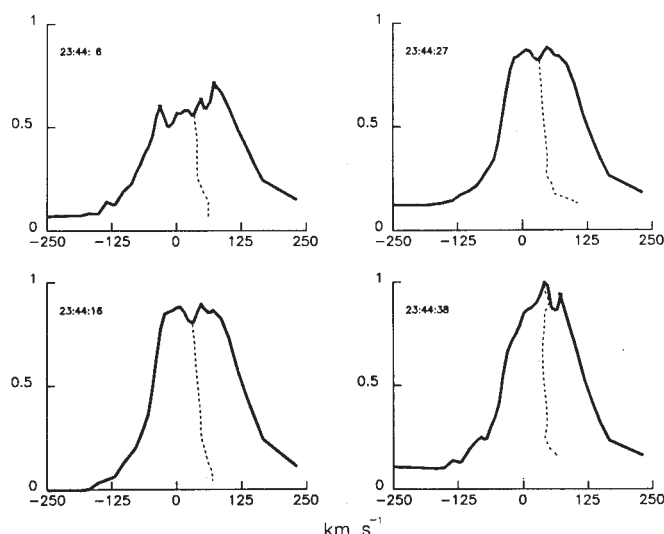


Fig. 2 SPO H α profiles in the brightest pixel, after subtraction of the preflare profile, showing a pronounced red asymmetry. Dotted lines indicate profile centroids. The shifts of the centroids in the extreme wings imply a peak condensation velocity for this pixel of about 50 km s^{-1} , falling by a factor of 2 on a timescale of about 30 s.

interpretation of observed impulsive phase X-ray blueshifts. Two of the competing interpretations are not consistent with our observations of this flare. In the magnetohydrodynamic model^{2,11} one expects both X-ray and H α material to be ejected outward, in contradiction to our present observation that they are oppositely directed. In the reconnecting-loop model², most of the X-ray emitting material is static, confined by a strong magnetic field to a low-lying high-pressure loop. It is easy to show¹ that the high coronal pressures created impulsively in the low-lying loop will drive extremely fast downflows ($\sim 200 \text{ km s}^{-1}$) in the H α -emitting chromosphere at the footpoints. Such dominance by downflowing H α -emitting material contradicts our present observation of oppositely directed flows carrying equal amounts of momentum. In the explosive chromospheric evaporation model⁸, the sudden creation of a high-pressure region at the footpoint of a coronal loop creates an opposite action and reaction, balancing upward and downward momentum. Of these three models, only the explosive chromospheric evaporation model predicts the observed equal momentum content of upflowing and downflowing material during the impulsive phase.

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- Antonucci, E. & Dennis, B. R. *Sol. Phys.* **86**, 67-77 (1983).
- Doschek, G. A. *et al. Chromospheric Explosions in High Energy Solar Phenomena* (eds Kundu, M. R. & Woodgate, B.) (NASA, in the press).
- Craig, I. J. D., McClymont, A. N. & Underwood, J. H. *Astr. Astrophys.* **70**, 1-9 (1978).
- Rosner, R., Tucker, W. H. & Vaiana, G. S. *Astrophys. J.* **220**, 643-665 (1978).
- Wolfson, C. J., Doyle, J. G., Leibacher, J. W. & Phillips, K. J. H. *Astrophys. J.* **269**, 319-328 (1983).
- Canfield, R. C. in *The Lower Atmosphere of Solar Flares* (ed. Neidig, D.) 10-24 (National Solar Observatory, Sunspot, 1986).
- Ichimoto, K. & Kurokawa, H. *Sol. Phys.* **93**, 105-121 (1985).
- Fisher, G. H., Canfield, R. C. & McClymont, A. N. *Astrophys. J.* **289**, 414-424 (1985).
- Fisher, G. H., Canfield, R. C. & McClymont, A. N. *Astrophys. J.* **289**, 434-441 (1985).
- Fisher, G. H. in *Lecture Notes in Physics* **255**, 53 (Springer, Berlin, 1986).
- Karpen, J. T., Doschek, G. A. & Seely, J. K. *Astrophys. J.* **306**, 327 (1986).

Mechanism for geomagnetic polarity reversals

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Recent studies of old magnetic observations¹ and the seismic velocity of the lower mantle² suggest that at least part of the geomagnetic secular variation is driven by temperature anomalies in the solid mantle³. In particular, a patch of flux of opposite sign to that expected for a dipole field occurs beneath southern Africa. It has intensified throughout the twentieth century, by a process that is believed to be flux expulsion associated with a particularly hot part of the lowermost mantle, and is drifting westwards. A similar patch lies beneath South America. Here I show that the present fall in the dipole moment is directly related to the intensification and southward movement of these patches and suggest the fall occasionally leads to polarity reversal. The almost linear increase in the frequency of polarity reversals that has occurred since the Cretaceous quiet interval is attributed to the growth of the hot patch of mantle presently under southern Africa.

A review of palaeointensity measurements⁴ has shown a clear fall in the dipole moment throughout the past 2,000 years; there is good evidence for growth in the preceding 2,000 years. Modern data also exhibit this fall in dipole moment: Fig. 1 shows the fall, in the twentieth century, of the Gauss coefficient g_1^0 , which is proportional to the dipole moment M

$$M = 4\pi a^3 g_1^0 / \mu_0 \quad (1)$$

where a is the Earth's radius. Clearly, recent secular variation data sample a small section of a long-term process of dipole decay, and we may hope to understand something of the mechanism of dipole decay by studying details of the present secular variation.

The fall in dipole moment has not been steady even in recent times. Figure 1 shows a rapid rate both now and earlier in the century, and a lower rate (15 nT yr^{-1}) around 1945. The field model for epoch 1842.5¹ suggests a fall at the lower rate for the second half of the nineteenth century, consistent with the palaeomagnetic results⁴.

How can this rapid fall in dipole moment be related to secular variation of the core field? At the core-mantle boundary g_1^0 is related to the radial field by an integral over the core surface

$$g_1^0 = (3c^3/8\pi a^3) \int Z \cos \theta dS \quad (2)$$

where c is the core radius, θ is colatitude and Z is the downward vertical component of the magnetic field. According to equation (2), a plot of $Z \cos \theta$ on the core-mantle boundary, in equal area projection, will indicate those features that contribute most to the dipole moment. Such a plot is shown in Fig. 2 using a field model for epoch 1945 derived by fitting a cubic time dependence through each Gauss coefficient of main field models (see ref. 1 and J. Bloxham, D.G. and A. Jackson, in preparation) for every decade from 1905.5 to 1955.5, 1966.0, 1969.5 and 1980.0. The dipole moment is proportional to the area average of the quantity plotted in Fig. 2. Clearly the main positive contributions come from the four features marked 1-4 called the 'main lobes' in the field by Bloxham and Gubbins¹. The only significant negative contributions come from features 5 and 6, the 'reverse flux' features, where radial field points in the opposite direction to that expected for a dipole (see Fig. 1 of ref. 1).

The main lobes have not moved substantially throughout historical time, but the reverse flux features have drifted westwards at rates of some tenths of a degree per year. The former may be associated with the main dynamo process⁶ whereas the

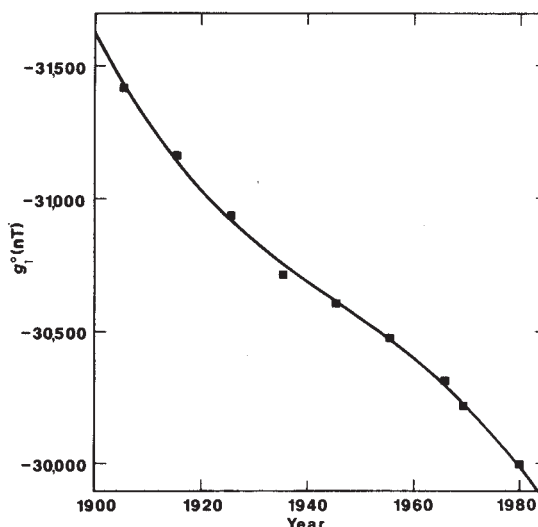


Fig. 1 Changes in the Gauss coefficient g_1^0 , which is proportional to the dipole moment, throughout the twentieth century. The points are values from core field models (ref. 1 and J. Bloxham, D.G. and A. Jackson, in preparation) the line is a best-fitting cubic used to calculate the main field and secular variation used in this paper (Figs 2 and 3).

latter appear to form near Indonesia and drift west to intensify strongly beneath southern Africa by expulsion of toroidal flux^{1,5}. Flux expulsion is believed to be caused by fluid upwelling associated with a region of hot mantle, a part of the mantle that has low seismic velocity². Mantle anomalies are stationary for long periods of time, and therefore associated drifting reverse flux patches will be a persistent feature of the secular variation throughout the recent geological past.

Changes in the dipole moment can be related to secular variation in the core field using the time derivative of equation (2) and a model for the secular variation. A model was obtained by differentiating the cubic fit to the main field models and truncating the result at degree 8. It compares well with other models based on observatory annual means⁷ but lacks their detail in the short-timescale features, such as jerks, which I wish to avoid here. Epoch 1945 was chosen to avoid inaccuracies in the cubic fit at the ends of the range.

Figure 3 shows that the fall in dipole moment results almost entirely from reverse flux features in the Southern Hemisphere—secular variation in the Northern Hemisphere (Fig. 3a) is low everywhere. A region of reverse flux can contribute to a change in dipole moment in two ways: either by intensifying *in situ* or by moving southwards into a region of larger $\cos \theta$. Feature 6 has intensified whereas feature 5, near South America, has moved south. The positive region of Fig. 3b is due mainly to the westward drift of feature 6.

In AD 1715, the earliest date for which core-field maps are available, feature 5 appeared in the central south Atlantic¹. Since then it has drifted westwards and increasingly southwards; its westward movement has been 35° of longitude in 265 yr ($0.13^\circ \text{ yr}^{-1}$). Feature 6, which is now beneath southern Africa, seems to have formed in the nineteenth century, drifted rapidly westward, and intensified in the early twentieth century. There is some evidence that it broke from an undulation in the magnetic equator beneath Indonesia in the late eighteenth century¹. The undulations are a persistent feature of the region and Gubbins and Bloxham¹ speculated that they are associated with core-mantle boundary topography, in which case they would be another long-term feature of the secular variation. If this identification is correct then feature 6 has drifted at a much faster rate than feature 5, by some 60° in 200 years ($0.3^\circ \text{ yr}^{-1}$); it must eventually catch up with feature 5 because of its faster rate of drift. Its greater speed can be seen in the models for the last

features will need a theoretical study. Flux patch 6 and the one to the north-west resemble sunspots and may have a similar origin⁵. The sunspot cycle has been modelled successfully with dynamo waves⁸ and a similar interpretation proposed for secular variation⁹. There are two distinct differences between these core spots and sunspots: the pairs form aligned north-west to south-east rather than laterally, which may indicate a weak toroidal field in the Earth's core¹⁰, and they propagate polewards rather than towards the Equator. Both differences may eventually be explained by a theoretical study including the dynamo itself—indeed poleward propagation is preferred in some theoretical models¹¹.

The dynamo wave theory is attractive in providing a link with reversals. Kinematic $\alpha\omega$ -dynamoes exhibit either steady or periodic reversal behaviour, depending on details of the dynamo model¹². The solar dynamo is well modelled by a periodic dynamo. The Earth may have a steady dynamo with superimposed waves driven by core-mantle boundary features such as topography beneath Indonesia or the hot region beneath southern Africa. At times of large amplitude the waves may induce a polarity reversal by a mechanism similar to that of a periodic $\alpha\omega$ -dynamo. The reversal could only occur occasionally when fluctuations led to an abnormally large set of reverse-flux features, as envisaged by Cox¹⁰.

Consider the consequences of this idea for geomagnetic polarity reversals. Each reversal results from secular variation features that are tied to the mantle. Local mantle temperature anomalies, like the one beneath southern Africa, change on the overturn time for mantle convection (~ 100 Myr). Therefore contemporaneous reversals should have similar characteristics (similar transition fields, for example). There is some palaeomagnetic evidence for this¹³. Any change in the mantle will lead to a change in secular variation characteristics, and consequently the nature and frequency of reversals.

At present the hot region at the base of the mantle lies in mid-latitudes at 40° S. A toroidal field in the core, generated by differential rotation acting on the predominantly dipolar field, will be strongest at mid-latitudes and zero at the poles and Equator^{14,15}; the hot region is therefore ideally located for expulsion of flux and production of reverse-flux features. This accounts for the present large variations in dipole moment and possibly for the high occurrence of reversals in the last 40 Myr. If the hot region had been weaker in the past, or located over the poles or some other region unfavourable for the production of toroidal field, then secular variation would have been lower and reversals less frequent.

Changes in the frequency of geomagnetic reversals are well documented¹⁶. The rate at which reversals occur has increased almost linearly¹⁷ during the past 86 Myr since the Cretaceous quiet interval when there were no reversals. It is suggested that this increase in reversal frequency is associated with the growth (or possibly the migration) of the hot region presently beneath southern Africa and the secular variation features it has driven.

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Analysis of multiexponential functions without a hypothesis as to the number of components

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The analysis of a curve as a sum of exponentials is one of the most common problems encountered in experimental 'curve-fitting' in such diverse areas as electrophysiology, chemical kinetics, electrical engineering and nuclear science. In this paper, a new method for the analysis of multicomponent exponential functions (either real and/or complex) is presented. This method is based upon the combined use of the Laplace transform and of Padé approximants. As compared with approximation procedures, it does not require an *a priori* hypothesis as to the number n of components, which is an output of the analysis¹. It thus becomes possible, under realistic numerical conditions, to address the problem of unambiguous detection of the exponential components. Performance comparisons show that several practical limitations reported in previous works are overcome. Detailed derivations relevant to the fundamental basis of the method described here are presented in ref. 2 and further developments pertaining to numerical analysis aspects may be found in ref. 13.

We shall consider in this paper functions of the form:

$$f(t) = \sum_{j=1}^n A_j e^{\mu_j t} \quad (1)$$

where, in the most general case, the μ_j are complex numbers

$$\mu_j = \lambda_j + i\omega_j$$

with i denoting the imaginary unit,

When the μ_j are purely imaginary ($\lambda_j = 0$), the problem of the extraction of the different periodic components from the sum (1) is solved by the use of the classical Fourier transform which generates n peaks corresponding in position and amplitude to the n components present in $f(t)$. Thus this method allows the detection of the periodic components with no *a priori* hypotheses as to the number n . If on the other hand we consider real μ_j values (with possibly damped oscillations for $\omega_j \neq 0$) the problem of components detection (similar on the surface to the previous one) has proved in actual practice to be much more difficult due to the sensitivity of the exponents and amplitudes to small changes in the data (in mathematical terminology these functions display non-orthogonal behaviour as compared to the case of purely periodic components). However, by analogy with Fourier's approach, numerous methods have been elaborated relying on the generation of peaks in spectral functions for the detection of the exponential components with real exponents. The general scope of these methods amounted in fact to performing (directly or indirectly) the inversion of the Laplace transform of $f(t)^{3-6}$. Severe limitations were however reported for these approaches, noticeably the presence of unresolved peaks for 'noisy' data. As a matter of fact, the practical difficulties encountered in the general problem of the evaluation of the inverse Laplace transform $\mathcal{L}^{-1}[f]$, for any $f(t)$, are now well-known⁷. Consequently, several workers have stated that the detection of exponential components inevitably leads to ill-posed problems and that methods for the analysis of functions such as (1) should address only the problem of function approximation. A function of unknown composition is approximated by k , $k+1 \dots$ exponentials and an 'optimal' value k_{opt} is determined, usually by statistical least-squares procedures. Now, Lanczos

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1. Bloxham, J. & Gubbins, D. *Nature* **317**, 77-781 (1985).
2. Dziewonski, A. M. *J. geophys. Res.* **89**, 5929-5952 (1984).
3. Bloxham, J. & Gubbins, D. *Nature* **325**, 511-513 (1987).
4. McElhinny, M. W. & Senanayake, W. E. *J. Geomag. Geoelect.* **34**, 39-51 (1982).
5. Bloxham, J. *Geophys. J. R. astr. Soc.* **87**, 669-678 (1986).
6. Gubbins, D. & Bloxham, J. *Nature* **325**, 509-511 (1987).
7. Langel, R. A., Kerridge, D., Barraclough, D. R. & Malin, S. R. C. *J. Geomag. Geoelect.* **38**, 573-597 (1986).
8. Steenbeck, M. & Krause, F. *Astr. Nachr.* **291**, 49-84 (1969).
9. Olsen, P. & Lee Hagee, V. *Geophys. J. R. astr. Soc.* **88**, 139-159 (1987).
10. Cox, A. *Rev. Geophys. Space Phys.* **13**, 35-51 (1975).
11. Glatzmaier, G. *Astrophys. J.* **291**, 300-307 (1985).
12. Roberts, P. H. *Phil. Trans. R. Soc.* **272**, 663-698 (1972).
13. Valet, J.-P. & Laj, C. *Nature* **311**, 552-555 (1984).
14. Elsasser, W. M. *Phys. Rev.* **69**, 106-116 (1946).
15. Bullard, E. C. & Gellman, H. *Phil. Trans. R. Soc.* **247**, 213-278 (1954).
16. Lowrie, W. & Kent, D. V. *Earth planet. Sci. Lett.* **62**, 305-313 (1983).
17. McFadden, P. L. & Merrill, R. T. *J. geophys. Res.* **89**, 3354-3362 (1984).

has provided examples in which a function sum of three exponentials could be reproduced to within two decimal places by a theoretical expression, a function sum of two exponentials, with significantly distorted time constants and amplitudes⁸. Following his pessimistic conclusions, it was generally accepted that an 'unrealistic accuracy' was required when using such procedures to recover the genuine exponential components.

The alternative method presented here allows the detection of exponential components with real and/or complex exponents under reasonable experimental conditions^{1,2}. We resort to the Laplace transform $\mathcal{L}[f]$ of $f(t)$, which is indeed associated in a natural way with exponential functions, instead of trying to solve the problem of the inversion of this transform. Rather, we recover the exponential components present in $f(t)$ by evaluating the analytic expression of the Laplace transform for a function $f(t)$ which is known only through numerical tabulated values.

The Laplace transform is expressed in the form:

$$\mathcal{L}[f](p) = \int_0^\infty e^{-pt} f(t) dt \quad (2)$$

where the variable p is a complex number. When applied to equation (1) this transformation leads to:

$$\mathcal{L}[f](p) = \sum_{j=1}^n \frac{A_j}{p - \mu_j} \quad (3)$$

If for an experimental function $f(t)$ known at sampled intervals $t_0, t_1, t_2, \dots$ the above analytic expression (with respect to p) of $\mathcal{L}[f]$ was available, the problem of the exponential components detection could be solved by a mere identification of the poles of the rational expression (3). In order to achieve this goal we first evaluate by numerical integration the value at some point p_0 of $\mathcal{L}[f](p)$, together with the values of its successive derivatives

$$\frac{d^j \mathcal{L}[f]}{dp^j}(p) = \int_0^\infty (-t)^j f(t) e^{-pt} dt$$

up to some order K , thereby obtaining a local representation of the Laplace transform at the point p_0 by the corresponding Taylor series truncated to the order K

$$\mathcal{L}[f](p) \approx \sum_{j=0}^K c_j (p - p_0)^j$$

with

$$c_j = 1/j! (d^j \mathcal{L}[f]/dp^j)(p_0)$$

The crucial point of the method is to derive the construction of the desired analytical representation through the use of Padé approximants⁹⁻¹¹. Padé approximants are well suited for this purpose, because according to equation (3) the Laplace transform is a rational function; in principle, therefore, it may be represented exactly in the whole complex plane by a Padé approximant. Hence we use the name 'Padé-Laplace' to designate this new method of analysis.

A Padé approximant of the variable p is a rational function denoted $[L/M](p)$ obtained by the division of two polynomials $A_L(p)$ and $B_M(p)$ of degree L and M respectively:

$$[L/M](p) = \frac{A_L(p)}{B_M(p)} = \frac{\sum_{s=0}^L a_s p^s}{\sum_{v=0}^M b_v p^v}$$

The basic feature of the Padé approximants which makes them appropriate for deriving an analytic expression for a function $F(p)$ knowing only a limited number of terms of its series expansion is that the power series expansion of the rational function $[L/M](p)$ is required to agree with that of $F(p)$ up to the term p^{M+L} . Accordingly, to achieve the construction of $[L/M](p)$, one needs the first $L+M+1$ coefficients of the Taylor series of $F(p)$. In a strictly theoretical frame, if the

Table 1 Analysis of a theoretical function $f(t)$

	Re μ_k	Im μ_k	Re A_k	Im A_k
Poles and amplitudes of Padé approximant [0/1]				
1	-0.00691	0.00000	185.15	0.00
Poles and amplitudes of Padé approximant [1/2]				
1	-0.00404	0.00000	150.93	0.00
2	-0.05271	0.00000	48.35	0.00
Poles and amplitudes of Padé approximant [2/3]				
1	-0.00400	0.00000	150.09	0.00
2	-0.04662	0.00000	40.09	0.00
3	-0.08651	0.00000	9.51	0.00
Poles and amplitudes of Padé approximant [3/4]				
a → 1	-0.00400	0.00000	150.00	0.00
b → 2	-0.04000	0.00000	19.01	0.00
c → 3	-0.06001	0.00000	29.99	0.00
d → 4	-0.40172	0.00000	1.00	0.00
Poles and amplitudes of Padé approximant [4/5]				
a → 1	-0.00400	0.00000	150.00	0.00
c → 2	-0.06001	0.00000	29.98	0.00
b → 3	-0.04001	0.00000	19.02	0.00
4	0.09178	0.00000	0.00	0.00
d → 5	-0.40240	0.00000	1.00	0.00
Poles and amplitudes of Padé approximant [5/6]				
a → 1	-0.00400	0.00000	150.00	0.00
b → 2	-0.04000	0.00000	18.99	0.00
c → 3	-0.05999	0.00000	30.01	0.00
4	0.00966	-0.04444	0.00	0.00
5	0.00966	0.04444	0.00	0.00
d → 6	-0.40038	0.00000	1.00	0.00

The analytic expression of the function analysed was: $f(t) = f_a(t) + f_b(t) + f_c(t) + f_d(t) = 150 \exp(-0.004t) + 19 \exp(-0.04t) + 30 \exp(-0.06t) + \exp(-0.4t)$ for $t = 0, 1, 2, \dots, 1199$. In order to be close to theoretical conditions (no external noise added and minimization of the computational noise) 1,200 points were generated on the computer and the values were rounded at the fifth decimal. The panel displays the output of the analysis in the form of the poles and the amplitudes of the Padé approximants $[(L-1)/L]$ of increasing order. For each approximant $[(L-1)/L]$ the parameters of the component k (with exponent μ_k and amplitude A_k) appear at the line whose index is k in the following order: Re μ_k , Im μ_k , Re A_k , Im A_k . As discussed earlier the effective parameters are obtained from the approximant [3/4]. The first artificial root appears in [4/5] at the rank $k=4$ with an amplitude of about 2.2×10^{-6} (this amplitude becomes zero with the number of digits chosen here for the table and is in a ratio of 45.5×10^4 with the smallest amplitude $A_d=1$ and in a ratio of 68.2×10^6 with the amplitude $A_a=150$). As a difference from the genuine roots this 'component' with a positive exponent is not stable and disappears in [5/6], splitting into a pair of complex conjugate roots with negligible amplitudes. Spurious roots which give in theory zero amplitudes correspond to an important theorem due to Padé and further developed by Chisholm and Baker^{15,16}. According to this theorem when the initial function is a rational function $F(p) = P_n(p)/Q_m(p)$ with n and m the degrees of the numerator and the denominator respectively, then the Padé approximant $[n/m]$ gives back exactly the initial function $F(p)$ and all higher order approximants $[N/M]$ (with $N \geq n$ and $M \geq m$) reduce to this $[n/m]$ exact expression. The example displayed allows comparisons with previous methods: (1) in the methods relying on the generation of peaks in spectral functions, the height of the peaks are governed by the values of the parameters A_k/μ_k which need to be in the same order of magnitude in order to get a satisfactory resolution⁴. We have chosen the inverse constraint as $A_d/\mu_d = 37,500$ and $A_d/\mu_d = 2.5$. The component d is very poorly taken into account in the data as its amplitude represents only 0.5% of the total while due to its very small time constant (100 times smaller than that of the component a) it decreases to 0 within very few time steps. These parameters originate from the analysis of correlation functions from patch-clamp data with the presence of components associated with very fast kinetics phenomena^{1,13} (manuscript in preparation). (2) The components b and c are such that $\mu_c/\mu_b = 1.5$. This ratio is much closer to the theoretical limit value 1 for the resolution of two exponentials than the value 2.5 reported by several workers, as a practical limit⁶. The calculation of the coefficients of the Padé approximants for a given set of c_j values was performed according to an algorithm of Longman¹⁷. For the calculation of the integrals c_j (see text) several methods were used and compared: a 48-point Gauss-Laguerre formula¹⁸, the trapezoidal rule and Simpson's integration rule which improved the efficiency of the method for analyses involving a large number of components or a high noise level (see Fig. 1). Recently we devised a variant of Simpson's rule after an idea of Filon^{19,20} and performed an analytic integration of the terms $(-t)^k \exp(-p_0 t)$ in the integrals c_j thus restricting the parabolic interpolation to the function $f(t)$ for which the Laplace transform is calculated. Another important point with integration methods such as Simpson's is the possibility of choosing a new time unit, thus getting a new value (denoted by ΔT) for the integration step. It actually appears that this choice is critical, the optimal value depending on the number N of points (ΔT was set to $1/\sqrt{N}$) (ref. 13), the technical details of which will be presented elsewhere.

Table 2 A famous example from Lanczos revisited

	Re μ_k	Im μ_k	Re A_k	Im A_k
Poles and amplitudes of the Padé approximant [0/1]				
1	-3.8617	0.0000	9.9407	0.0000
Poles and amplitudes of the Padé approximant [1/2]				
1	-1.7241	0.0000	1.5489	0.0000
2	-4.5867	0.0000	8.4504	0.0000
Poles and amplitudes of the Padé approximant [2/3]				
$a \rightarrow 1$	-0.9962	0.0000	0.4015	0.0000
$b \rightarrow 2$	-3.0162	0.0000	3.4435	0.0000
$c \rightarrow 3$	-5.0086	0.0000	6.1550	0.0000
Poles and amplitudes of the Padé approximant [3/4]				
1	1.3183	0.0000	0.0000	0.0000
$a \rightarrow 2$	-0.9593	0.0000	0.3711	0.0000
$b \rightarrow 3$	-2.9593	0.0000	3.3361	0.0000
$c \rightarrow 4$	-4.9872	0.0000	6.2929	0.0000
Poles and amplitudes of the Padé approximant [4/5]				
1	6.5216	0.0000	0.0000	0.0000
2	2.3722	0.0000	0.0000	0.0000
$a \rightarrow 3$	-0.9826	0.0000	0.3893	0.0000
$b \rightarrow 4$	-2.9914	0.0000	3.3925	0.0000
$c \rightarrow 5$	-4.9986	0.0000	6.2182	0.0000

We consider the function $f(t) = A_a \exp(-t) + A_b \exp(-3t) + A_c \exp(-5t)$ originally presented by Lanczos⁸. We normalize parameters by setting $A_a + A_b + A_c = 10$, multiplying each A_i by a factor of about 4: $A_a = 0.4$, $A_b = 3.4$ and $A_c = 6.2$. We mimicked Lanczos' conditions by generating 24 sampled $f(t)$ values rounded to the second decimal, with the only difference being that the sampling step is set to 0.15 instead of 0.05 in order to ensure a full decay to 0 for $f(t)$. The output of the computer analysis shows with no ambiguity that in our framework the procedure of detection is effective. The approximation with two exponentials provided by the approximant [1/2] allows a reconstitution of the initial data with a standard root mean square deviation of 0.01. It was in fact the possibility of such a fit to the data with two exponential components, with distorted parameters, which was the basis of Lanczos' pessimistic conclusion. With such sparse data (the time constants are of the order of magnitude of the sampling step) the numerical integration in the Laplace transformation would introduce too large an error due to the parabolic interpolation. The analysis shown was thus performed with one of the variants allowed by the generalization of the framework provided by our method. We define a functional transformation well-adapted for the identification of some type of components f_i . In the logic of our approach such a transformation $\mathcal{T}[f]$ (with $f = \sum_i f_i$) should allow the detection of the components f_i as poles of the analytic expression of $\mathcal{T}[f]$, derived from its Taylor series expansion by use of Padé approximants (appendix by P.C. in ref. 1) (ref. 2). Here, a well-adapted transformation is the z -transform which may be viewed as a discrete version of the Laplace transform. The well-known Prony method for the analysis of multiexponential functions can be reformulated as a [Padé]-[z -transform] method with the constraint that the parameter z_0 for which the Taylor series expansion is calculated is placed at infinity²¹. For the above example, with this choice for z_0 , the Padé approximants of successive orders yielded a representation with two exponentials whose parameters are in agreement with those of the approximation with 2 exponentials provided by Lanczos. In marked contrast, with a suitable finite value for z_0 (here $z_0 = 2$), the three genuine components are recovered in the analysis displayed in table at orders higher than [1/2] (the approximation with two exponentials obtained at [1/2] is again essentially equivalent to the one proposed by Lanczos). This feature clearly illustrates the increase in detection power gained through the allowance for an optimal choice for the value of z_0 (or, correspondingly, for the parameter p_0 in the Padé-Laplace procedure).

original function $F(p)$ is a rational function of degree $N-1$ for the numerator and N for the denominator (as in the function $\mathcal{L}[f](p)$ in equation (3) after reduction to the same denominator), then the corresponding functions $[L/M](p)$ will be 'approximants' only up to order $[(N-2)/(N-1)]$, because the Padé approximant $[(N-1)/N]$ will in fact give the exact analytic expression of $F(p)$.

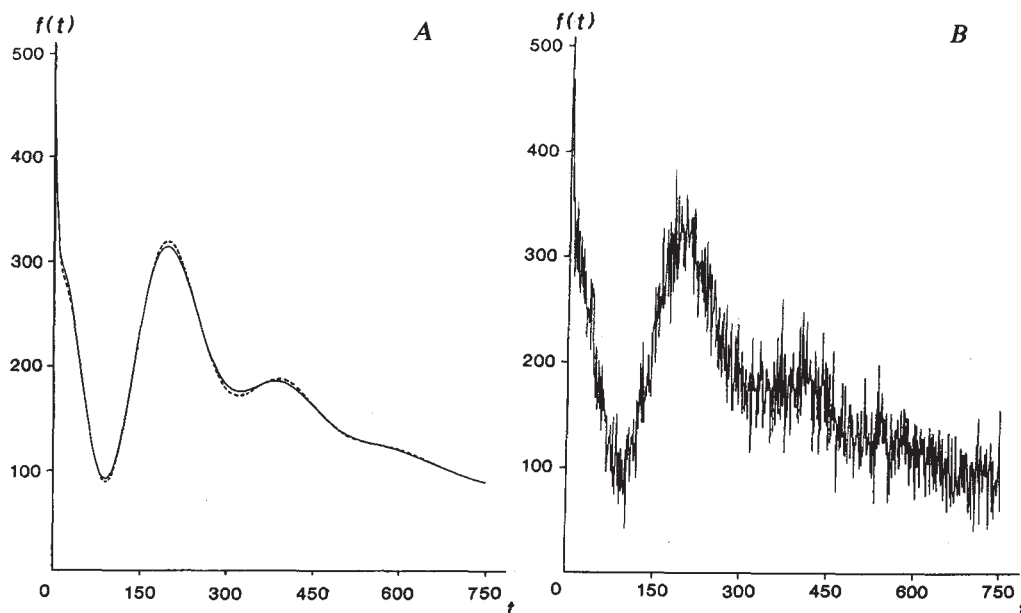
In order to illustrate the way in which the components detection is achieved through this procedure, let us first consider a theoretical function $f(t)$, sum of four exponentials (Table 1, which also gives complementary information relevant to aspects of our numerical analysis). We consider the successive Padé approximants $[(L-1)/L]$ resulting from a number of Taylor coefficients of the Laplace transform of $f(t)$. First, the Padé approximant [0/1] provides (through its pole and the corresponding residue) an approximation of the original function with a single exponential; second, the Padé approximant [1/2] provides (through its two poles obtained as roots of the denominator and the corresponding residues) an approximation

Table 3 Padé-Laplace analysis of the curves in Fig. 1

	Re μ_k	Im μ_k	Re A_k	Im A_k
Poles and amplitudes of Padé approximant [3/4]				
1	-0.00222	0.00000	464.24	0.00
2	-0.00837	0.00000	-261.70	0.00
3	-0.00553	-0.02580	97.43	-71.65
4	-0.00553	0.02580	97.43	71.65
Poles and amplitudes of Padé approximant [4/5]				
$a \rightarrow 1$	-0.00200	0.00000	400.00	0.00
$c \rightarrow 2$	-0.02000	0.00000	-500.00	0.00
$b \rightarrow 3$	-0.00800	-0.03000	175.00	0.00
$d \rightarrow 4$	-0.00800	0.03000	175.00	0.00
$d \rightarrow 5$	-0.20002	0.00000	250.02	0.00
Poles and amplitudes of Padé approximant [5/6]				
$a \rightarrow 1$	-0.00200	0.00000	400.00	0.00
$c \rightarrow 2$	-0.02000	0.00000	-500.01	0.00
3	-0.04614	0.00000	0.03	0.00
$b \rightarrow 4$	-0.00800	-0.03000	175.00	0.00
5	-0.00800	0.03000	175.00	0.00
$d \rightarrow 6$	-0.20004	0.00000	250.01	0.00
Poles and amplitudes of Padé approximant [8/9]				
1	0.00995	0.00000	0.00	0.00
$a \rightarrow 2$	-0.00200	0.00000	400.00	0.00
$c \rightarrow 3$	-0.02000	0.00000	-500.00	0.00
$b \rightarrow 4$	-0.00800	-0.03000	175.00	-0.01
5	-0.00800	0.03000	175.00	0.01
6	0.00019	-0.01785	0.00	0.00
7	0.00019	0.01785	0.00	0.00
$d \rightarrow 8$	-0.19998	0.00000	249.86	0.00
9	-0.61936	0.00000	0.55	0.00
Poles and amplitudes of Padé approximant [10/11]				
$a \rightarrow 1$	-0.00200	0.00000	398.92	0.00
2	0.00209	-0.01182	-0.03	0.00
3	0.00209	0.01182	-0.03	0.00
$c \rightarrow 4$	-0.02074	0.00000	-512.85	0.00
$b \rightarrow 5$	-0.00800	-0.02995	177.79	-0.45
6	-0.00800	0.02995	177.79	0.45
7	0.01191	-0.03198	0.00	0.00
8	0.01191	0.03198	0.00	0.00
9	0.00322	-0.05576	-0.04	-0.22
10	0.00322	0.05576	-0.04	0.22
$d \rightarrow 11$	-0.19600	0.00000	264.66	0.00
Poles and amplitudes of Padé approximant [11/12]				
1	0.00122	0.00000	-0.09	0.00
$a \rightarrow 2$	-0.00201	0.00000	401.67	0.00
3	0.00348	-0.02315	0.02	0.00
4	0.00348	0.02315	0.02	0.00
$c \rightarrow 5$	-0.02009	0.00000	-506.57	0.00
6	0.00039	-0.00929	0.06	0.12
7	0.00039	0.00929	0.06	-0.12
$b \rightarrow 8$	-0.00791	-0.03004	174.41	1.58
9	-0.00791	0.03004	174.41	-1.58
10	0.01047	-0.06043	0.04	-0.05
11	0.01047	0.06043	0.04	0.05
$d \rightarrow 12$	-0.19738	0.00000	262.22	0.00

of the original function with two exponentials; the Padé approximant [3/4] gives the exact theoretical expression of the original function. It is evident that in this analysis the series of roots in the successive denominators will not display any common component. Because, in general, one will not know the expression of the original function, and not even the number of components of this expression, one must pursue this analysis further. However, for the specific example considered it will be sufficient to obtain the [4/5] approximant, because it must reduce to the [3/4] approximant, and this will remain true for all approximants of higher orders. That is, the [4/5] approximant will display with no modifications the four components already detected in the [3/4] approximant, and also an additional component—which may be called an 'artificial component' (see caption of Table 1)—corresponding to the multiplication of the numerator and the denominator by an equal factor. This component will have a zero amplitude A_i in the partial fraction expansion (3). In conclusion, if one is interested only in obtaining an approximation of the function analysed with L components, one may restrict the analysis to the $[(L-1)/L]$ approximant. On the other hand, if the problem of detection is

Fig. 1 Application to simulated data with gaussian noise (sum of exponentials with real exponents and one cosine component). The analytical function $f(t)$ generated on the computer was $f(t) = f_a(t) + f_b(t) + f_c(t) + f_d(t) = 400 \exp(-0.002t) + 350 \exp(-0.008t) \cos(0.03t) - 500 \exp(-0.02t) + 250 \exp(-0.2t)$ and is represented in panel A (solid line). Gaussian noise with mean zero and a standard deviation value of 25 was added to $f(t)$, the resulting 'noisy function' is represented in B. The level of noise (ratio of the standard deviation value to the amplitude of each component) ranged between 5% (component c) and 10% (component d). The components detection was first performed on the data without noise (3,000 sampled points for $t = 0, 1, 2, \dots$; value chosen for the integration step $\Delta T = 0.01826$; $p_0 = 0.89$) and the resulting output is displayed in Table 3 (approximants [3/4] to [8/9]). The 4 components labelled a, b, c and d are detected in the approximants [4/5] and [5/6]. The first artificial root which appears in [5/6] has an amplitude of the order 3×10^{-2} . It should be noted that using the complex exponential notation, the component $f_b(t)$ is displayed as the sum of two conjugate roots $f_b(t) = 175 \exp(-0.008t) [\exp(i0.03t) + \exp(-i0.03t)] = 350 \exp(-0.008t) \cos(0.03t)$. The resulting curve is drawn in a and is indistinguishable from the theoretical curve $f(t)$. In order to demonstrate the stability of the analysis, an approximant of high order ([8/9]) is also displayed. The quality of the analysis was slightly affected when a severe truncation was performed, taking into account only the 750 points represented in a (for example, the amplitude for $f_c(t)$ was -499.45 against -500). The output of the analysis performed on the data with noise in the same conditions as for the curve without noise is also shown in Table 3 (approximants [10/11] and [11/12]). The four components are again detected, the slight numerical alteration affecting mainly the component d having the smallest amplitude. The curve corresponding to the analytical expression derived from approximant [11/12] (Table 3) is drawn in A (dashed). The analysis and detection were also efficient for gaussian noise having a standard deviation of 50 with, however, a rather significant alteration for the amplitude of the component $f_a(t)$ (334 against 250).



to be solved with no *a priori* hypotheses as to n , a step-wise procedure would be adopted, in which the Padé approximants of increasing orders are considered, the detection being achieved when this procedure is pursued up to order $[L/(L+1)]$ (at which one 'artificial root' appears for the first time). At that point, the previous approximant $[(L-1)/L]$ is equal to the exact Laplace transform $[(n-1)/n]$, and it therefore gives the desired exponents and amplitudes. The numerical realization of this theoretical scheme is illustrated by the computer output given in Table 1. On the grounds of performance comparisons for theoretical functions, the example analysed shows also that severe limitations reported in several previous works for the separation and detection of exponential components are overcome by the procedure. Theoretical comparisons with other methods can be found in ref. 2.

Next, we further assess the practical effectiveness of the detection procedure in more realistic cases. First, it was worth revisiting in Table 2 the famous example of Lanczos, already mentioned above⁸, which led the author to the extremely pessimistic conclusion (reiterated ever since by researchers in the field) that the components separation problem is so 'skew-angular' and encounters 'such unsurmountable difficulties' that the only remedy would be an 'increase of accuracy' to 'completely unrealistic' limits⁸. It would not be advisable in a genuine experimental case to address the identification problem with such poor information (as for the function in Table 2 derived from Lanczos' treatment) nevertheless, we believe that this example allows a significant shift in the limits which had to be set for the validity of such analyses.

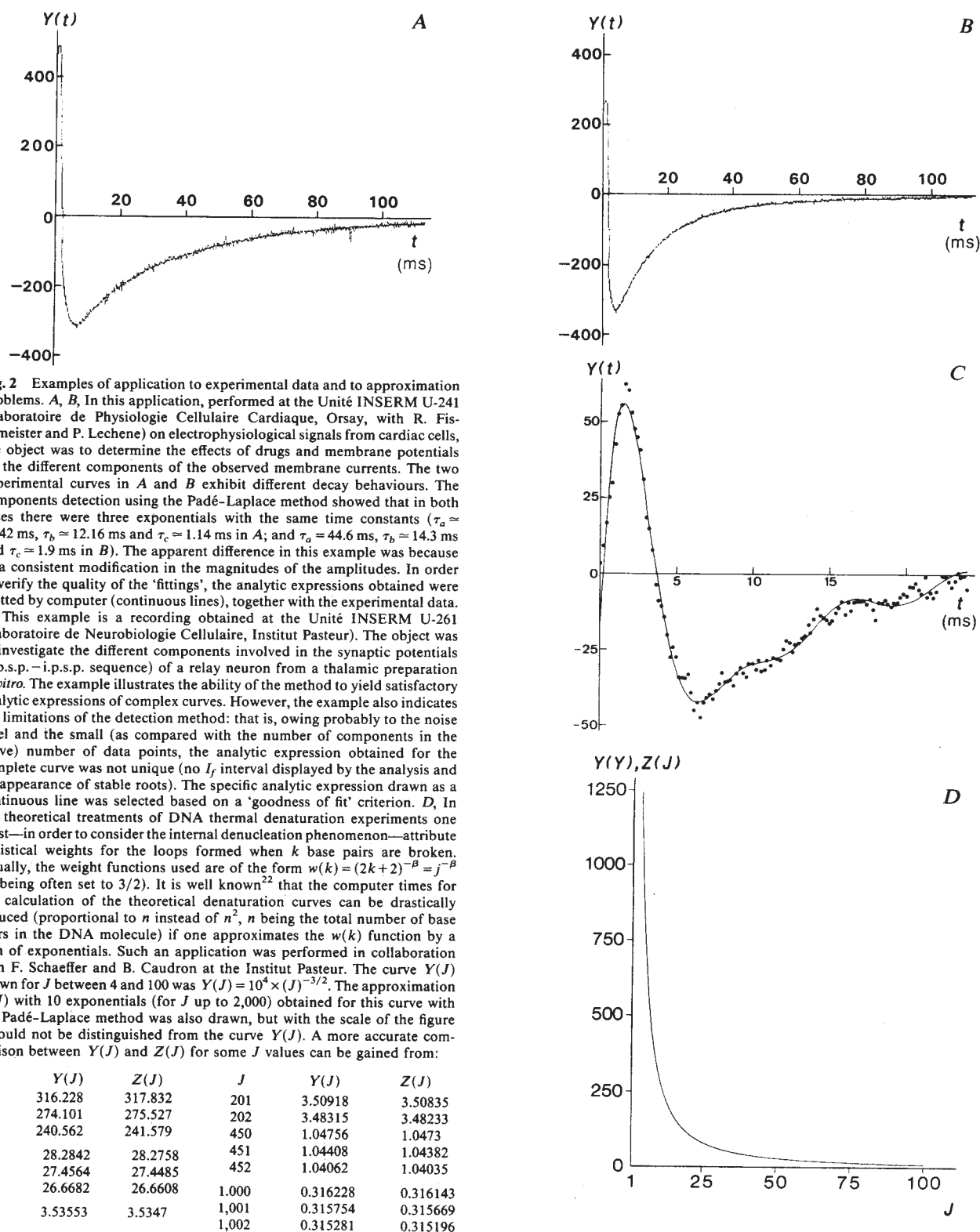
This example, through a comparison with a well-known method for the analysis of multiexponential functions, namely the one proposed by Prony in 1795¹², also highlights an essential practical feature of our approach. In a strictly theoretical framework, the detection procedure described is effective for any p_0 value. However, numerical investigations indicated that in practice the identification of components could be performed only for p_0 values lying in some 'optimal' interval, denoted by I_f (refs 2 and 13). In fact, it is not surprising that the value chosen for p_0 (which is the only input parameter of the method)

can significantly affect the numerical precision in the calculation of the integrals c_j defining the coefficients of the Taylor series expansion of $\mathcal{L}[f]$. We examined the theoretical and numerical aspects of this problem, in order to implement an automatic procedure of analysis¹³. It appears that the optimal choice for p_0 is one such that the coefficients c_j are of the same order of magnitude. The general idea is thus to set (by varying the free parameter p_0) the centre of the convergence disk of the Taylor series of $\mathcal{L}[f]$ at a distance equal to 1 from the pole μ_1 of the Laplace transform which lies closest to 0. It is clear that on the other hand extreme p_0 values will lead to loss of computational accuracy. The detailed criterion and the iterative automatic procedure elaborated for the choice of p_0 will be discussed elsewhere.

Table 3 illustrates the effect of the addition of artificial gaussian noise to an analytic function. The data show that, despite high noise levels (see Fig. 1 for a graphic representation of this level), the components detection and analysis still perform satisfactorily (Table 3). The example treated also demonstrates the ability of the method to detect accurately the complex components.

In Fig. 2, two experimental applications of the method are displayed. In the example shown in Fig. 2d, the method (as a method of approximation) was deliberately applied to a curve which is not a sum of exponentials, namely $Y(J) = J^{-3/2}$, in order to obtain an analytic expression of this curve with the desired number of exponential components. Other applications include the analysis of chemical relaxation signals¹⁴ and electrophysiological signals from cardiac cells (manuscript in preparation, see Fig. 2).

In conclusion, in each case of practical application, numerical simulations such as those discussed above could allow an assessment of the limits of validity of the detection procedure for exponential components (the limit of resolution of the exponents for a given accuracy or noise level). Also from a practical point of view, it becomes possible to implement on-line procedures which do not need the technical skill demanded by many sophisticated nonlinear least-squares procedures. From the theoretical point of view a rational generalization of the framework



elaborated² could allow one to address various problems in the field of models identification, such as the detection of lorentzian or gaussian components and possibly global analyses of certain two-dimensional data (by use of two-dimensional Padé approximants). Work in these fields is in progress.

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1. Yeramian, E. *Problème de l'indépendance ou du couplage fonctionnel des récepteurs d'acétylcholine—Mise au point d'une nouvelle méthode (transformation intégrale, approximations de Padé) pour l'analyse des fonctions sommes d'exponentielles réelles ou complexes* Rapport de stage (Ecole Centrale des Arts et Manufactures, Châtenay-Malabry, 1981).

2. Claverie, P. & Denis, A. *Computer Phys. Rep.* (in the press).
3. Gardner, D. G., Gardner, J. C., Lausch, G. & Meinke, W. W. *J. chem. Phys.* **31**, 978-986 (1959).
4. Smith, M. R., Cohn-Sfetcu, S. & Buckmaster, H. A. *Technometrics* **18**, 467-482 (1976).
5. Provencher, S. W. *J. chem. Phys.* **64**, 2772-2777 (1976).
6. Stockmann, H. J. *Nucl. Instrum. Meth.* **150**, 273-281 (1978).
7. Bellman, R., Kalaba, R. E. & Lockett, J. A. *Numerical Inversion of the Laplace Transform: Applications to Biology, Economics, Engineering and Physics* (Elsevier, New York, 1966).
8. Lanczos, C. *Applied Analysis*, 272-280 (Prentice-Hall, Englewood Cliffs, 1957).
9. Baker, G. A. Jr *Adv. theor. Phys.* **1**, 1-58 (1965).
10. Baker, G. A. Jr & Grave-Morris, P. R. *Encyclopaedia of Mathematics and its Applications* Vols 13, 14 (Addison-Wesley, Massachusetts, 1981).
11. Gilewicz, J. *Approximants de Padé (Lecture notes in Mathematics No. 667)* (Springer, Berlin, 1978).
12. Prony, R. *J. Ecole Polytechnique* **1**, 24-76 (1795).
13. Yeramian, E. thesis, Ecole Centrale des Arts et Manufactures, Chatenay-Malabry (1986).
14. Aubard, J. Levoir, P., Denis, A. & Claverie, P. *Computers Chem.* (in the press).
15. Chisholm, J. S. R. in *Padé Approximants* (ed. Graves-Morris, P. R.) 1-18 (The Institute of Physics, Bristol, 1973).
16. Baker, G. A. Jr *J. Math. Anal. Appl.* **43**, 498-528 (1973).
17. Longman, I. M. *Int. J. Computer Math.* **B3**, 53-64 (1971).
18. Stroud, A. H. & Secrest, D. *Gaussian Quadrature Formulas* (Prentice-Hall, Englewood Cliffs, New Jersey, 1966).
19. Filon, L. N. G. *Proc. R. Soc. Edin.* **XLIX**, 38-47 (1928-29).
20. Tranter, C. J. *Integral Transforms in Mathematical Physics* (Chapman and Hall, London, 1971).
21. Weiss, L. & McDonough, R. N. *SIAM Rev.* **5**(2), 145-149 (1963).
22. Fixman, M. & Juan, J. J. *Biopolymers* **16**, 2693-2704 (1977).

New explanation for chain folding in polymers

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Polymer crystals are characteristically thin in the molecular direction¹ so that materials such as polyethylene and nylon have an extremely finely divided texture consisting of crystal lamellae interspaced with non-crystalline regions (melting points are depressed by 20 °C or more). Many distinctive properties of these polymers follow as a consequence of this phenomenon, often known as chain folding because molecules generally fold back and forth across the thin dimension of the crystals. The currently accepted explanation of the origin of chain folding in terms of 'nucleation' ('LH' theories²) appears to be inconsistent with the observed shapes of crystals³ (see also Wunderlich⁴). In this paper I describe how an approximate treatment of an alternative model^{5,6} involving rough growth surfaces and molecular 'pinning' can give analytical results in line with experiment. The aim is to derive the result in such a way as to concentrate on the physical mechanisms involved and to discuss the kinetic barriers caused by molecular continuity. If there turns out to be any connection between chain folding of synthetic molecules and folding in proteins, it will be clear that the models discussed here may be more readily extended to the latter than LH models², because LH models² are specific to crystals and not applicable, for example, to the ribbon structures which are formed from β sheets^{7,8}.

Polymer crystals are generally platelets (Fig. 1a and b). The thickness l is typically 4-120 lattice repeats (~ 5 -50 nm) and only slightly greater than l_m , the thickness that would be in equilibrium with the liquid at the crystallization temperature

$$l_m = \epsilon T_m^0 / h \Delta T \quad (1)$$

$\epsilon/2$ is the (fold) surface free energy per chain emerging at the fold surfaces, h is the latent heat of fusion per unit, T_m^0 the melting point of a crystal of infinite thickness, and ΔT the supercooling; l is measured in numbers of units each of several chemical repeats^{3,9}. The concept of a unit allows in a simple, if non-rigorous way, for the connectedness of the monomers, such that they cannot move independently, and also for any stiffness of the chain. It follows from equation (1), and from l generally being close in value to l_m , that $l \sim \Delta T^{-1}$, the commonly observed trend^{10,11}.

A kinetic theory for chain folding is one that predicts lamellae

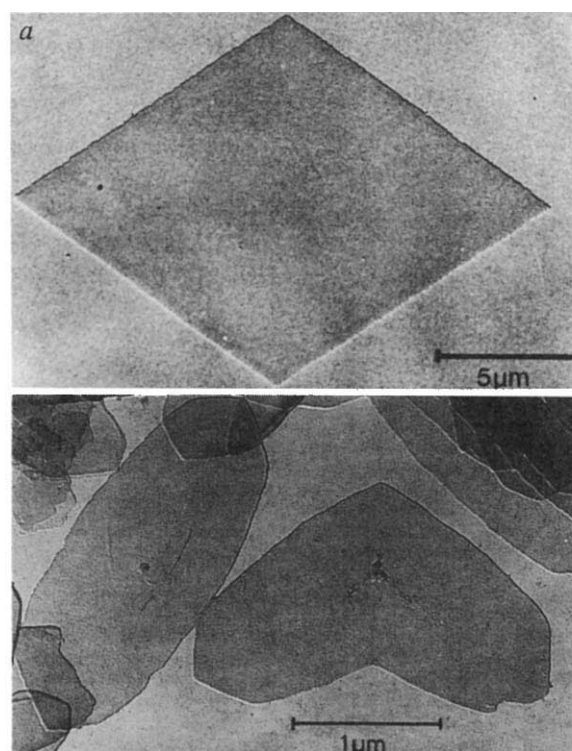


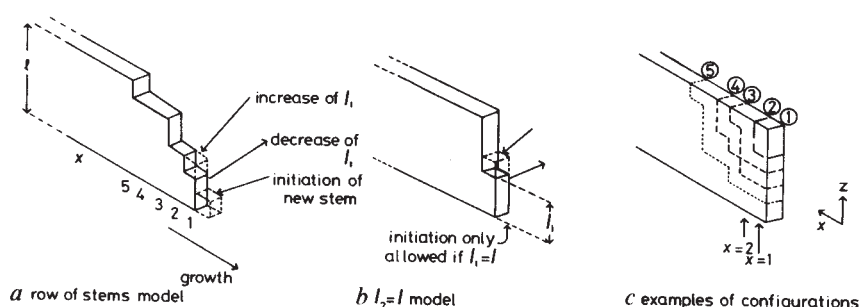
Fig. 1 a, Electron micrographs of polyethylene crystals, showing straight and curved shapes of outlines. In the latter case the crystals must have re-entrant corner sites on the perimeter (for example, at twin boundary of the twinned crystal), (courtesy of Dr S. Organ). Curved rather than straight outlines tend to occur for higher crystallization temperatures, which suggests a molecular roughness on the perimeter which increases with temperature³. b, Schematic representation of a polymer crystal indicating the chain direction, fold and growth surfaces, and the nature of a growth face step as envisaged by nucleation (LH) theories. The inset shows an alternative view of the region of the growth face involving a step^{3,5}. In which case the stems at the growth face can be short. The details of the folding back (adjacent re-entry or otherwise²⁸) are not of primary interest here: several types of folds and loops are included. The dotted lines refer to the structures in Fig. 2.

as the morphology corresponding to fast growth, so that the final state is generally metastable and not in equilibrium. We now consider why the speed of a growth front (growth rate G) for a given l value can then be written according to the following equation, for both nucleation and roughness-pinning models^{12,13}

$$G \propto e^{-Kl(l-l_m)\Delta f/kT} \quad (2)$$

Δf is the (bulk) change in free energy on crystallization ($h\Delta T/T_m^0$). The following elucidates how equation (2) is equivalent to those usually given for LH theories^{2,9,14-22,29} where

Fig. 2 Representations of the two-dimensional models discussed in this paper (see dotted lines in Fig. 1b). *a*, The row of stems model, with molecular direction (*z*) vertical as in Fig. 1b. Vertical 'bond' energies ϵ can be associated with loops and partially attached chains (for example). Modification to the lengths of stems are only permitted at the outermost end of the row (that is, $x=1$, to the right). The length l_1 of the outermost stem may be increased or decreased by one unit. Stems at $x=1$ of length unity can be removed, and initiation of a new stem of one unit can be made adjacent to the previous outermost stem. The length l_2 of the next but one outermost stem can only be changed if the stem at $x=1$ is removed. *b*, In the $l_2=l$ model, only those configurations of the row of stems model are allowed where l_2 (the length at $x=2$) is equal to the final length l . These configurations are 'viable' for growth, whereas those in *a* with $l_2 < l$ are not, because they must remove at least one stem before the configuration can evolve into one with all the stems in this part of the row being of length l . *c*, Examples of stem configurations, labelled 1–5. Configuration 1 (probability $PC(l)$) is the squared-off (non-tapered) one through which the system must pass before the stem in the position currently labelled $x=1$ can be incorporated in the body of the row crystal. Configurations 1 and 2 are described within the $l_2=l$ model (total probability P for all such). Configurations 3–5 are such as to block the movement of the growth point. The energies increase with the number of short stems. For example, 5, (dotted line) would have a relatively high energy and low probability. The total number of configurations, each one weighted according to its Boltzmann probability, can be calculated using a random walk analogy (derivation of equation (4)). The principal assumptions and approximations of the row of stems model used here are given in the text and their limitations and justifications are discussed in more detail elsewhere. A list of these is as follows: neglect of correlations along the growth face between different rows; contributions to energy are accounted for by nearest neighbour 'bonds' in a two-dimensional lattice; the molecular continuity is accounted for by pinning rules (changes only at $x=1$); l is a uniform in fully grown row, $l_{x+1} \geq l_x$; the distribution of l_x near the growing end corresponds to equilibrium. The last approximation is made for mathematical simplicity only (see note in proof).



the 'barrier term' e^{-Kl} arises as the probability of overcoming the (largely enthalpic) free energy barrier involved with the creation of steps on the growth face. A step (Fig. 1b) has an associated free energy σ_n which, in LH theories, contains the product $\sigma a l$ where σ is a side-surface free energy per area and $a l$ is the area exposed in the step. The crucial assumptions are: (1) σ_n increases linearly with l and (2) σ_n is generally large compared with kT . Growth rates are then calculated¹⁸ as the product of the steady-state density of steps and the average rate at which steps spread. The exponent Kl is either $2\sigma_n/kT$ or σ_n/kT , depending on the substrate width (regimes I or II^{19,18}). The term in equation (2) proportional to $(l-l_m)$ can be derived from the probability that patches can be removed, and from the step spreading rate. Such a term is clearly necessary to ensure $G=0$ at the melting point ($l=l_m$). Equation (2) gives a maximum in G with respect to l ¹⁵ (also see below in connection with Fig. 3), and as long as K is sufficiently large this maximum corresponds to l not much greater than l_m . The term e^{-Kl} for $l=l_m$, with l_m substituted from equation (1), then becomes $e^{-K_g T \Delta T}$ (K_g being a constant) which has the same dependence on ΔT as is found for G experimentally¹⁴ (the dependence of G on ΔT is dominated by the barrier term e^{-Kl}). There have been recent developments in LH theories² (for example refs 20–22), but they all retain the two basic assumptions concerning σ_n . For K to be sufficiently large for l and G values to be derived in this way, large σ_n values are essential (for example, 25 kT for polyethylene lamellae of thickness 30 nm). For any such high σ_n , re-entrant corner sites on the crystal perimeter should be very much favoured for addition of new material, (for example, by factors of $e^{2\sigma_n/kT} \approx e^{50}$ or more in polyethylene) because attachments elsewhere involve the creation of two steps with a consequent free energy barrier of $2\sigma_n$. Hence all crystal shapes should evolve very quickly during growth to give straight-sided convex polygons. In contrast, crystals with curved perimeters^{3,23} and double crystals and twins with re-entrant corner sites²⁴ are commonly observed morphologies that are stable during growth (see also Fig. 1a). The assumption $\sigma_n \propto l$ in LH theories² is also at variance with estimates of σ_n from crystal shapes. In several cases where information is available, thicker lamellae tend to show more curvature than thin ones (for example, the lower part of Fig. 1a compared with the upper part). Hence, as curvature implies lower σ_n , morphology then suggests that σ_n tends to decrease (not increase) with l .

It has been shown that curved crystal outlines can be the

consequence of rough growth faces²⁵. If indeed there can be such a roughness for polymer crystals³ there must be a barrier to the growth which is completely different from nucleation. The following represents a very simple form of the model, and shows how a barrier term of the form e^{-Kl} can arise. This is because in the model there is an entropic barrier to growth. The growth face explores many configurations, only a minority of which allow the face to progress. Most are in effect blocked by the kinetic constraints imposed by molecular connectivity.

Consider the row of stems model where a stem is a sequence of polymer chain which traverses the crystalline core of the lamella (Fig. 1b). Consider a row of stems perpendicular to the growth face which grows at its end: (one is dotted in Fig. 1b). A growth face in Fig. 1b consists of a parallel array of ends of rows. Each stem is made up of units so that the row consists of a two-dimensional array of units with a reduction in energy ϵ associated with notional 'bonds' between any adjacent units (intrastem along z and interstem along x , see Fig. 2 and legend). Units are added or removed, with probabilities governed by rate constants which depend on the appropriate Boltzmann factors^{5,6}. Molecular continuity is included by a simple additional rule: units are only free to add or subtract at the extremity of the row ($x=1$, Fig. 2a)—elsewhere the stems are deemed to be pinned. The ends of pinned stems correspond to folds (or molecular ends). If the row of stems did in fact represent an isolated entity (for example, β sheet) this rule would be the natural consequence of the molecule pleating back and forth within the row. For applications to three-dimensional crystals the rule allows for the increase in probability of stems participating in folds the further back they are from the growth front. This model is sufficient to account for the variations $l \sim \Delta T^{-1}$ and $G \sim e^{-K_g T \Delta T}$ (either using a rate equation approach⁶ or computer simulation⁵).

The following simplified treatment of the row of stems model contains the essence of the argument. It is assumed here that all the stems in the completed row have the same length l and that $l_x \leq l_{x+1}$ where l_x is the length of the x th stem ($x=1$ is at the growing end and $x=2$ corresponds to the stems next to the end, see Fig. 2a). Consider firstly the model where $l_2=l$; this restriction is equivalent to limiting the initiation of a new stem to configurations where $l_1=l$ (Fig. 2b). The growth rate G' for this model is readily obtained by considering the concentrations $C(i)$ of various $l_1=i$ states, and the rates of conversion between them (as for other sequential processes). State $i=0$ is taken to

be equivalent to $i = l$. For example, state $l_1 = l$ can either change to state $l_1 = 1$ (by addition of a new stem with a total probability of $C(l)k^+$ where k^+ is the arrival rate of units to the row) or to state $l_1 = l-1$ (by removal of a unit from the stem, with a probability of $k_1 C(l)$ where $k_1 = k^+ k = k^+ e^{-\Delta f/kT}$ is the rate constant for stem reduction). The rate constant for stem removal, $C(1)$ state to $C(l)$, k_2 , is $k^+ e^{(\varepsilon - \Delta f)/kT}$. The result is l simultaneous equations; for example, one such is obtained by equating creation and removal rates for state $l_1 = 1$ ($C(l)k^+ + C(2)k_1$ and $C(1)k^+ + C(1)k_2$ respectively). The values of the $C(i)$ (and hence the fluxes) can be obtained by solving these equations. As expected, G' depends essentially linearly on temperature (D. M. Sadler, in preparation)

$$G'/k^+ = (1 - k_1^{(l-l_m)})/D \approx (l - l_m)\Delta f/kTD \quad (\text{as } l \rightarrow l_m) \quad (3)$$

D depends on k^+ , k_1 , k_2 and l is of the order of l^2 , and is equal to l^2 in the limit $h \ll kT$. The probability $C(l)$, that $l_1 = l$ for the $l_2 = l$ model, can also be calculated and is of the order l^{-1} .

In the more general case of the row of stems model, the configurations with $l_2 = l$ exist as a minority. All the other configurations are not 'viable', in the sense that at least some units must be removed before the process of initiating and lengthening of stems can proceed indefinitely to give a uniform l value in the body of the 'crystal'. These removal events are interpreted as allowing the removal of folds or loops unfavourable to growth. The barrier term arises from the probability P that configurations are viable ($l_2 = l$).

The probability that $l_1 = l$ in the row of stems model is equal to $PC(l)$ and can be derived using the approximation¹³ that the distribution of stem lengths is the same as in equilibrium (that is, for zero growth, with a value of ΔT adjusted so that $l = l_m$). The state $l_1 = l$ has zero energy for the case $l = l_m$, so that its Boltzmann weighting factor in calculating a partition function is unity. A simple mathematical device for enumerating the weighted sum over all configurations (the partition function) is to consider random walks: each walk corresponds to a configuration (for example, as in Fig. 2c). The weighting is achieved by making the ratio of probabilities for horizontal displacements to vertical ones in the walk equal to k_1^{l-z} where z is the height along the stem. The conditions for random walks apply because this ratio depends only on z and not on the previous history of any particular walk. The probability of an initial vertical displacement is unity (the walk must start somewhere) so the probability of $l-1$ successive vertical displacements to give $l_1 = l$ leads to

$$PC(l) = \pi_{z=l}^{-1} \{1/(1 + k^{(l-z)})\} \quad (4a)$$

This result is closely related to an exponential dependence on l . This is shown by simplifying equation (4a) for two limiting cases, according to the magnitude of the largest of the terms $k^{(l-z)}$.

$k^l \approx 1$ means that all the $k_1^{(l-z)}$ are close to unity and

$$PC(l) \approx 2^{-l+1} \quad (4b)$$

$k^l \approx 0$ and large l . In this case the product is converted into a sum by taking logarithms, and the sum is approximated as an integral

$$\ln(PC(l)) \approx -\int_1^{l-1} \ln(1 + k^m) dm$$

A substitution $u = k^m$ then gives

$$PC(l) \approx e^{-\pi kT/12\varepsilon} \quad (4c)$$

ε appears in the result because, for $l = l_m$, $\varepsilon = l\Delta f$ (from equation (1)).

G is the product of P and G' , which are decreasing and increasing functions of l respectively (see the solid lines in Fig. 3 and equation (2)). G has a maximum at l slightly greater than l_m (dashed line in Fig. 3). Just as for the LH theory¹⁵ this is what is required for a kinetic theory to predict chain folding. The derivation of an l value is often made by averaging over such a growth rate, which implies an ensemble of crystals each of uniform l . A second approach^{15,16} allows for fluctuations in

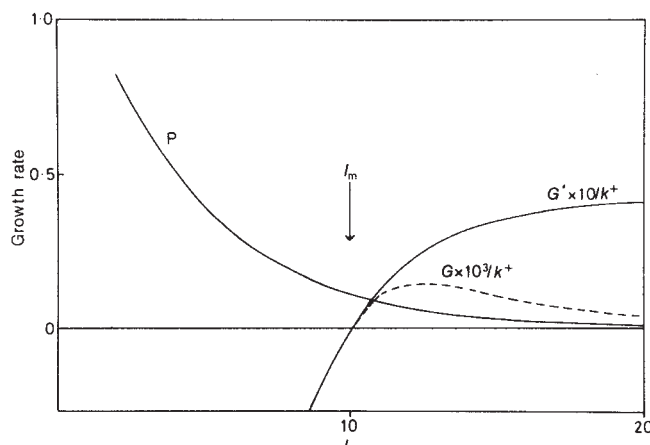


Fig. 3 The growth rate G in numbers of new stems per unit time, dashed line, for the row of stems model (with all the stems in the fully grown rows being of length l). The contributions to this growth rate are P , the probability that a row is viable (see Fig. 2b), and G' , the growth rate for viable rows. P and G' were calculated from equations (3) and (4a), with $\Delta T/T_m^0 = 0.05$, $\varepsilon = 3 kT_m^0$, $h = 6 kT_m^0$. (See Fig. 1 of Frank and Tosi¹⁵ for the LH model.) G/k^+ and G'/k^+ are normalized growth rates.

thickness within each crystal. For the mathematically analogous case of the row of stems model, the numerical solution of the complete set of rate equations⁶ allows for fluctuations, and the average l value obtained is less than would be obtained from averaging over the dashed curve¹³. This is because there is an energetic barrier to fluctuating upwards in stem length which is absent for fluctuating downwards.

The interpretation of the (largely entropic) barrier to growth which exists in the row of stems model is that most stem configurations are tapered; these do not allow the growth front to progress, because in doing so short stems would be engulfed in the body of the crystal and average l values would become $< l_m$. The growth tip constantly explores the available configurations, and when a non-tapered one is reached the front can advance. Equation (4) gives the probability of such situations arising, and shows that it is an exponentially decreasing function of l : hence it is a barrier term e^{-Kl} in equation (2). In applying this to polymer crystals, the rule that changes only occur at the outermost part of the crystal follows from stems elsewhere being pinned, for example by folds.

The exponents in equation (4) can be compared with K_g from experimental plots of $\ln(G)$ against $1/T\Delta T$ (ref. 14), using the accepted estimates of experimental parameters¹⁴, for example, the fold surface free energy which can be used to relate $l(l - l_m)$ to ΔT^{-1} . The only free parameter is the average number n_u of monomers in a unit, and equating theoretical and observed exponents for equation (4b) gives $n_u \sim 3$ for polyethylene. This probably represents a satisfactory numerical agreement in growth rates considering the simplicity of the model (n_u is by its nature a rather ill-defined quantity; $n_u \approx 10$ has been suggested^{15,9}). Changes in exponent (for example, in going from equation (4b) to (4c)) may relate to observed changes in K_g which have hitherto been associated with regime changes^{19,20}.

A growth surface roughness which increases with T (ref. 3) is also consistent with a marked increase in G with T when data for the same ΔT are compared^{14,26,27}.

In conclusion, observation shows that in general re-entrant corners on the crystal growth faces only have a minor influence on the growth rate. Corner (step) creation, 'nucleation', is not therefore the barrier that leads to the distinctive lamella nature of polymer crystals. An alternative kinetic model, based on rough growth surfaces and the kinetic restrictions resulting from molecular connectivity (pinning), appears to be compatible with many experimental observations.

I thank Dr G. H. Gilmer for collaboration on related work which overlaps the present one, and Professor A. Keller and Dr H. D. Keith for assessments of the manuscript.

Note added in proof: It has recently been found that the rates of growth and primary nucleation of long chain paraffins can show minima as a function of temperature³⁰. Such an effect suggests an entropy barrier to growth and has been seen in calculations using the row model. The minima correspond to cases where there are significant departures from 'equilibrium' as regards the distribution of l_x , in contrast to the approximation used in the derivation of equation (4a) (Sadler and Gilmer, to be published).

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- Keller, A. *Phil. Mag.* **2**, 1171-1175 (1957).
- Lauritzen, J. I. & Hoffman, J. D. *J. Res. natn. Bur. Stand.* **64A**, 73-102 (1960).
- Sadler, D. M. *Polymer* **24**, 1401-1409 (1983).
- Wunderlich, B. *Discuss. Faraday Soc.* **68**, 239 (1979).
- Sadler, D. M. & Gilmer, G. H. *Polymer* **25**, 1446-1452 (1984).
- Sadler, D. M. & Gilmer, G. H. *Phys. Rev. Lett.* **56**, 2708-2711 (1986).
- Pitts, U. B. & Finkelstein, A. V. *Q. Rev. Biophys.* **13**, 339-386 (1980).
- Atkins, E. D. T. *Discuss. Faraday Soc.* **68**, 409-411 (1979).
- Point, J. J. *Macromolecules* **12**, 770-775 (1979).
- Kawai, T. & Keller, A. *Phil. Mag.* **11**, 1165-1177 (1965).
- Barham, P. J., Chivers, R. A., Keller, A., Martinez-Salazar, J. & Organ, S. J. *J. mater. Sci.* **20**, 1625-1630 (1985).
- Sadler, D. M. *J. Polym. Sci., Phys. Ed.* **23**, 1533-1554 (1985); *Polymer Commun.* **27**, 140-145 (1986); *J. chem. Phys.* (in the press) *Polymer* (submitted).
- Sadler, D. M. & Gilmer, G. H. *Phys. Rev.* (in the press).
- Hoffman, J. D., Davis, G. T. & Lauritzen, J. I. in *Treatise on Solid State Chemistry* (ed. Hannay, N. B.) 497-614 (Plenum, 1976).
- Frank, F. C. & Tosi, M. *Proc. R. Soc. Lond.* **263A**, 323-339 (1961).
- Hoffman, J. D. et al. *Kolloid Zeitschrift für Polymere* **231**, 564-592 (1969).
- Lauritzen, J. I., Passaglia, E. & DiMarzio, E. A. *J. chem. Phys.* **45**, 4444-4454 (1966).
- Frank, F. C. *J. Cryst. Growth* **22**, 233-236 (1974).
- Lauritzen, J. I. *J. appl. Phys.* **44**, 4353-4359 (1973).
- Hoffman, J. D. *Polymer* **24**, 3-26 (1983).
- Hoffman, J. D. *Polymer* **23**, 656-670 (1982).
- Hoffman, J. D. *Polymer* **26**, 803-810; 1763-1778 (1985).
- Keith, H. D. *J. Appl. Phys.* **35**, 3115-3126 (1964).
- Sadler, D. M., Barber, M., Lark, G. & Hill, M. J. *Polymer* **27**, 25-34 (1986).
- Avron, J. E. et al. *Phys. Rev. Lett.* **45**, 814-817 (1980).
- Sadler, D. M. *Polymer Commun.* **25**, 196-201 (1984).
- Organ, S. & Keller, A. *J. Polym. Sci., Phys. Ed.* **24**, 2319-2335 (1986).
- Sadler, D. M. in *Structure of Crystalline Polymers* 125-180 (ed. Hall, I.) (Applied Science, Barking, 1984).
- Mandelkern, L., Fatou, J. G. & Howard, C. *J. Chem. Phys.* **69**, 956-959 (1965).
- Ungar, G. & Keller, A. *Polymer* (submitted).

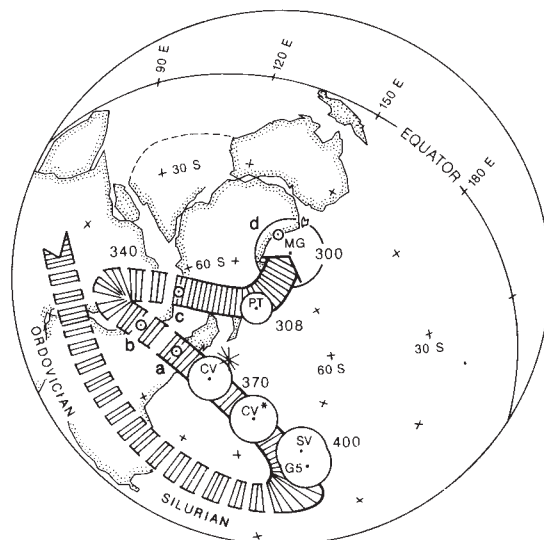


Fig. 1 Revised apparent polar wander path (APWP) for Gondwanaland¹² based on new Devonian poles from Australia (ref. 4 and P. W. Schmidt, personal communication) and reinterpretation of some existing palaeopole positions as likely to be mid-Carboniferous*. Palaeomagnetic poles shown are: early Devonian—G5 (ref. 6), SV (Snowy River Volcanics) (P. W. Schmidt, personal communication), taken as 400 Myr; late Devonian—CV and CV* (ref. 4) (370 Myr); late Carboniferous—PT (ref. 34) (308 Myr) and MG (ref. 34) (300 Myr). The Ordovician and Silurian part of the APWP is consistent with Goleby's⁶ and Morel and Irving's⁵ interpretation of Gondwanan palaeopoles. In the figure a–d correspond to the palaeopoles used for the 360 to 300 Myr palaeogeographical maps (Fig. 1).

Namurian uplift in Australia and South America triggered the main Gondwanan glaciation

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The Permo-Carboniferous glacial sediment at the base of the Gondwanan basins reflects the culmination^{1,2} of the glacial episode that started in Australia and southern South America in the Namurian, after two local episodes in northern South America and adjacent Africa in the Visean and latest Devonian³ (Fig. 1). Here we show that the start of each episode was associated with orogenic uplift of the bordering regions: northern South America and adjacent Africa in the latest Devonian and Visean, and southern South America and eastern and central Australia in the Namurian. All areas, except southern South America in the Namurian, were at mid- (and not high) latitudes. Thus, we show that whereas the first order control of glaciation was the configuration of the continents and oceans during the closing of Palaeo-Tethys, orogenesis provided a second order control by the accumulation of ice about bordering uplifts during the three glacial episodes.

The late Palaeozoic glaciation of Gondwanaland (Fig. 1) comprised three episodes: (1) in the latest Devonian in northern South America and adjacent Africa³; (2) in the Visean in Brazil³; and (3) starting in the Namurian in southern South America and eastern and central Australia, extending into the other

Gondwanaland continents during the Permo-Carboniferous (300–290 Myr BP) culmination. A refined apparent polar wander path (APWP) for Australia, based on palaeomagnetism⁴ (Fig. 2), enables us to determine more accurately than previously the timing of changing palaeolatitudes in Gondwanaland during the late Palaeozoic glaciation. This APWP confirms earlier determinations^{5,6} of the pole in the Palaeo-Pacific and its return to Gondwanaland at the end of the Devonian; but, contrary to the premise that glacial centres developed on land in high or polar latitudes³, we find that the latest Devonian and Visean glacial episodes of South America and the Namurian episode of Australia are mid-latitudinal (30°–60°). During the Permo-Carboniferous culmination, glaciation was polar in Antarctica and Australia but ranged from 60°–20° S in the rest of Gondwanaland. The onset of each glacial episode is therefore not attributable to polar latitude; rather, each glacial episode started during times of orogenic uplift in the bordering regions. For the latest Devonian glacial episode, the uplifts accompanied the latest Devonian deformation of the northern and central Andes⁷ and of the Mauritanides⁸; for the Visean episode, the uplifts are indicated by the deposition of piedmont conglomerate in the Ambo Group of Bolivia⁹.

The earliest Namurian age of the onset of the third glacial episode is indicated by deposits in eastern Australia and southern South America. The oldest record of Palaeozoic ice at or near sea level in Australia is earliest Namurian (333 Myr), as shown directly by the presence of striated stones in the *Cravenoceras*-bearing Kullatine Formation¹⁰ and in the piedmont deposit of the Spion Kop Conglomerate¹¹. At the same time, a rapid cooling of sea water is indicated by a sharp fall in the diversity of brachiopods¹², the first appearance of the endemic Gondwana fauna¹³, and the last appearance of oolite¹⁴. The striated stones have been regarded as reflecting an alpine glaciation only^{1,15}, but we see now that the uplift that shed the Spion Kop Conglomerate was not a narrow mountain chain but instead extended across the 1,000 km-wide Tasman Fold Belt

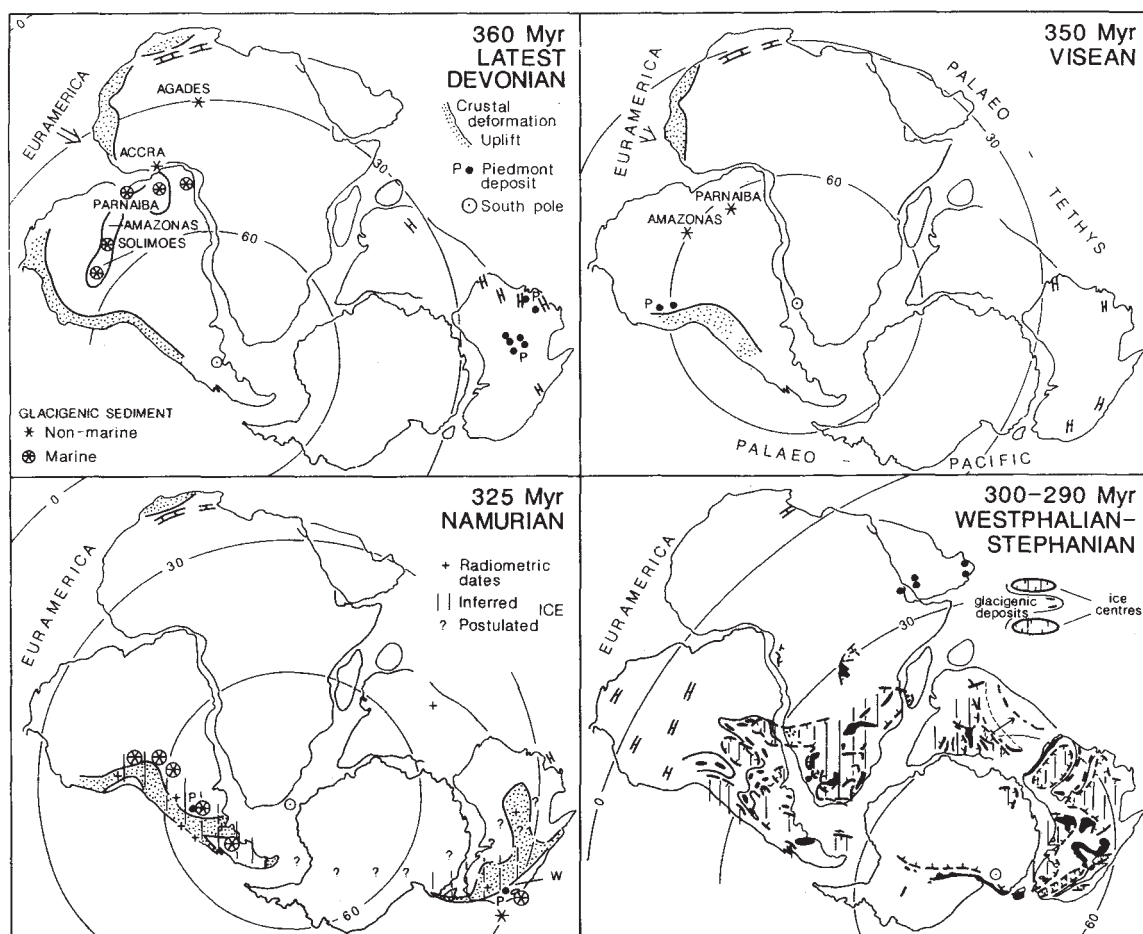
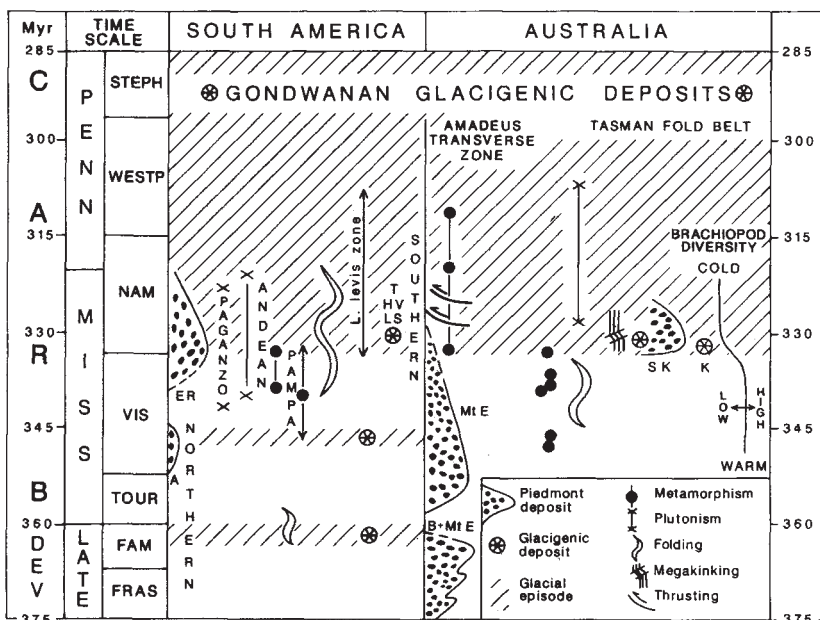


Fig. 2 Palaeogeography of Gondwanaland at 360, 350, 325, and 300–290 Myr, showing latitude (from Fig. 2), metamorphic and plutonic radiometric dates (from Fig. 3), deformation and uplift^{7,8,19–21}, piedmont deposits^{9,11}, glacigenic deposits^{15,11,19,31}, inferred and postulated ice and carbonates. Gondwanaland reconstruction³² with northern margin of Greater India shown palinspastically by removing the Cenozoic rotational underthrusting³³, indicated at 300 Myr by the arrow.

Fig. 3 Timetable³⁵ of events in South America and Australia during the late Devonian and Carboniferous. Shown for northern South America are the latest Devonian folding and uplift⁷, of the same age as that of the Mauritanides⁸, both concurrent with glaciation³, and Visean uplift, indicated by the Ambo Group (A) of Peru⁹, concurrent with glaciation in Brazil³. Shown for southern South America are the Namurian piedmont El Raton Formation (ER), plutonism in the Paganzo³⁶ and Andean^{21–24} regions, metamorphism in the Andean²¹ and Pampa^{22–24} regions, folding²¹, the range of the *Levipustula levis* zone (ref. 9), and the estimated age of the glacigenic sediments of the Las Salinas (LS), Hoyada Verde (HV), and Tarija (T) Formations (refs 25, 26). Younger glacigenic sediments up to the Westphalian/Stephanian culmination not shown. For Australia are shown the events in (1) the Amadeus Transverse Zone: the piedmont deposits of the Brewer Conglomerate (B) of the Amadeus Basin¹⁷ and the Mount Eclipse Sandstone (Mt E) of the Ngalia Basin (ref. 18 and personal information), metamorphism dated by the Rb/Sr ages^{37,38} of micas generated during retrogressive metamorphism of Proterozoic rocks, and thrusting^{18,37}, and (2) the Tasman Fold Belt: metamorphism^{39,40}, plutonism (S. E. Shaw, personal communication), folding and megakinking^{16,41}, the piedmont and glacigenic Spion Kop Conglomerate¹¹ (SK), the glacigenic Kullatine Formation¹⁰ (K), and the sharp fall in diversity of brachiopods, interpreted as a change from warm to cold¹², which coincides with the first appearance of the Gondwanan fauna¹³ and the last appearance of warm-water oolite¹⁴.



and Victoria Land, and through the Amadeus Transverse Zone of central Australia (Fig. 1, 325 Myr). In the Tasman Fold Belt, Visean (340 ± 10 Myr) deformation and metamorphism by east-west (Palaeo-Pacific) shortening was followed in the Namurian by north-south compression that produced megakinks and the intrusion of granitoids, while the Proterozoic basement and Adelaidean and Palaeozoic cover of the intracratonic Amadeus Transverse Zone underwent metamorphism during crustal shortening of 100 to 150 km by overthrusting and the growth of nappes¹⁶. Precursor uplifts in the latest Devonian produced 3-km thick piedmont deposits in Central Australia^{17,18}, but the more intense Namurian deformation there lacks equivalent piedmont deposits. The sediments expected to have been shed from the uplifts, then nunataks, were postulated to have been incorporated in the ice and transported to the marine edge of the sheet to be deposited as dropstones, or to have been protected in the ice until its decay in the Stephanian¹⁹. An alternative possibility is that glacial deposits were reworked into the base of the Permo-Carboniferous basins so that only a few, if any, deposits were preserved.

We have since found that the mid-Carboniferous uplift can be extended to Victoria Land²⁰ and, more importantly, on the other side of Gondwanaland, to the Palaeo-Pacific margin of South America. Here a major deformation ('Eohercynian Phase', ref. 21) in the Andean region and in the northern Patagonian region of Argentina²² is dated by syntectonic metamorphism and plutonism^{21,23,24}, and is accompanied and followed by rapid uplift as shown by the Namurian²⁵ piedmont El Raton Formation. The glacial marine Hoyada Verde Formation of the Calingasta/Uspallata Basin²⁶, the Las Salinas Formation of the Central Patagonian Basin²⁶, and the Tarija Formation of Bolivia⁹ lie within or below the *Levipustula levis* zone. The overlap of the Mississippian plant *Archeosigillaria converta* in the *L. levis* zone of the Las Salinas Formation²⁵ indicates that the glacial sediments were deposited in the lower or Namurian part of the range of this zone. These events show that South America's development in the Namurian paralleled that of Australia (Fig. 3): orogenic deformation accompanied by uplift and glaciation²⁷. That the glaciation was substantial is shown by glacio-eustatic drawdown in Euramerica at the start of cyclothemic deposition²⁸.

The plate-tectonic control of the configuration of the continents and oceans, in particular the late Palaeozoic closing of Palaeo-Tethys by the joining of Gondwanaland and Euramerica^{2,29} and the attendant reduced rate of subduction and lowered concentration of atmospheric CO₂ (ref. 30), is taken as the first order cause of the late Palaeozoic glaciation. Gondwanaland drifted beneath the South Pole in the late Devonian, thus making it susceptible to glaciation, yet widespread glaciation did not start until the Namurian, and then it was mid-latitude except in southern South America. What distinguished the Namurian was extensive deformation and uplift in two widely separated parts of Gondwanaland. The correlation in space and time of orogenic uplift with the two preliminary glaciations, and with the Namurian part of the main glaciation, thus leads us to conclude that orogenesis provided the trigger for the late Palaeozoic glaciation.

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1. Frakes, L. A. *Climates Throughout Geologic Time* (Elsevier, Amsterdam, 1979).
2. Crowell, J. C. *Am. J. Sci.* **278**, 1345-1372 (1978).
3. Caputo, M. V. & Crowell, J. C. *Geol. Soc. Am. Bull.* **96**, 1020-1036 (1985).
4. Schmidt, P. W., Embleton, B. J. J., Cudahy, T. J. & Powell, C. McA. *Tectonics* **5**, 135-150 (1986).
5. Morel, P. & Irving, E. J. *Geol.* **86**, 535-561 (1978).
6. Goleby, B. R. *J. Geomagn. Geoelect. Suppl. III* **32**, 11-21 (1980).

7. Harrington, H. J. in *Encyclopedia of World Regional Geology, Part 1, Western Hemisphere* (ed. Fairbridge, R. W.) 456-465 (Dowden, Hutchinson & Ross, Stroudsburg, 1975).
8. Lecorche, J.-P. in *Regional trends in the geology of the Appalachian-Caledonian-Hercynian-Mauritanide Orogen* (ed. Schenk, P. E.) 347-353 (Reidel, Dordrecht, 1983).
9. Martinez Diaz, C. (ed.) *The Carboniferous of the World, II* (Inst. Geol. y Minero de Espana, 1985).
10. Campbell, K. S. W. *J. Paleont.* **36**, 38-52 (1962).
11. Hambrey, M. J. & Harland, W. B. (eds) *Earth's Pre-Pleistocene Glacial Record* (Cambridge University Press, 1981).
12. Roberts, J. *Lethaia* **14**, 123-134 (1981).
13. Runnegar, B. & Campbell, K. S. W. *Earth Sci. Rev.* **12**, 235-257 (1976).
14. Campbell, K. S. W. & McKellar, R. G. in *Stratigraphy and Palaeontology, Essays in Honour of Dorothy Hill* (ed. Campbell, K. S. W.) 77-119 (Australian National University Press, Canberra, 1969).
15. Crowell, J. C. & Frakes, L. A. *J. geol. Soc. Aust.* **17**, 115-155 (1971).
16. Powell, C. McA. *Geology* **12**, 546-549 (1984).
17. Jones, B. G. *J. geol. Soc. Aust.* **19**, 229-249 (1972).
18. Wells, A. T. & Moss, F. J. *Bull. Bur. Miner. Resour. Geol. Geophys. Aust.* **212**, (1983).
19. Powell, C. McA. & Veevers, J. J. in *Phanerozoic Earth History of Australia* (ed. Veevers, J. J.) 348-350 (Clarendon, Oxford, 1984).
20. Grindley, G. W. & Oliver, P. J. in *Antarctic Earth Science* (eds Oliver, R. L., James, P. R. & Jago, J. B.) 133-139 (Australian Academy of Science, Canberra, 1983).
21. Dalmayrac, B., Laubacher, G., Marocco, R., Martinez, C. & Tomasi, P. *Geol. Rdsch.* **69**, 1-21 (1980).
22. Ramos, V. A. *9th Congreso Geol. Argentino Actas* **2**, 311-325 (1984).
23. Ramos, E. D. & Ramos, V. A. *7th Congreso Geol. Argentino, Actas* **1**, 771-786 (1979).
24. Linares, E., Llabias, E. & Latorre, C. O. *Revta Asoc. geol. Argent.* **35**, 87-146 (1980).
25. Azcuy, C. L., Cesari, S. N. & Longobucco, M. I. *Ameghiana* **38**, 11-28 (1981).
26. Gonzalez, C. R. in *Tills and Related Deposits* (eds Evenson, E. B., Schuchter, C. & Rabassa, J.) 271-277 (Balkema, Rotterdam, 1983).
27. Frakes, L. A. & Crowell, J. C. *Geol. Soc. Am. Bull.* **80**, 1007-1042 (1969).
28. Veevers, J. J. & Powell, C. McA. *Geol. Soc. Am. Bull.* (in the press).
29. Ross, C. A. & Ross, J. R. P. *Geology* **13**, 27-30 (1985).
30. Fischer, A. G. in *Catastrophes and Earth History* (eds Berggren, W. A. & van Couvering, J. A.) 129-150 (Princeton University Press, 1984).
31. Braakman, J. H., Levell, B. K., Martin, J. H., Potter, T. L. & van Vliet, A. *Nature* **299**, 48-50 (1982).
32. Powell, C. McA., Johnson, B. D. & Veevers, J. J. *Tectonophysics* **63**, 13-29 (1980).
33. Klootwijk, C. T., Conaghan, P. J. & Powell, C. McA. *Earth planet. Sci. Lett.* **75**, 167-183 (1985).
34. Irving, E. J. *geophys. Res.* **71**, 6025-6051 (1966).
35. Harland, W. B. et al. *A Geologic Time Scale* (Cambridge University Press, 1982).
36. McBride, S. L., Caelles, J. C., Clark, A. H. & Farrar, E. *Earth planet. Sci. Lett.* **29**, 373-383 (1976).
37. Armstrong, R. L. & Stewart, A. J. *J. geol. Soc. Aust.* **22**, 103-115 (1975).
38. Black, L. P. & Gulson, B. L. *BMJ J. Aust. Geol. Geophys.* **3**, 227-232 (1978).
39. Shaw, S. E., Flood, R. H. & Riley, G. H. *J. geol. Soc. Aust.* **29**, 41-48 (1982).
40. Wyborn, D. & Owen, M. *Bur. Miner. Resour. Geol. Geophys. Aust.* 1:100,000 Geological Map Series, Araluen, New South Wales, Sheet 8826 (1983).
41. Powell, C. McA., Cole, J. P. & Cudahy, T. J. *J. struct. Geol.* **7**, 281-300 (1985).

Bacterioplanetol from chemical degradation of an oil shale kerogen

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Microbial lipids play an important role in the formation of geologically derived organic matter¹. Among these lipids the hopanoids have a key function as they show an almost ubiquitous occurrence in sediments and fossil fuels². In this report we have obtained bacterioplanetol, the well-known presumptive precursor of most fossil hopanoids, by catalytic hydrogenolysis of the Messel oil shale kerogen (Eocene, 50 × 10⁶ yr). The existence of chemically bonded biohopanoids in an Eocene kerogen supports the idea of incorporation of intact biological structures into high molecular weight organic matter of geological origin. Selective chemical degradation is, therefore, a valuable tool in the structural characterization of polymeric organic matter in the sedimentary environment.

Extensive research has been conducted since the first discovery and identification of geohopanoids³. Investigations have concentrated on the stereochemical transformations of this compound class in order to obtain maturity indicators for organic matter⁴. Although the biological precursors of fossil hopanoids were unknown for a long time, their distributions have intensive geochemical application in basin studies or pollution studies of Recent sediments^{5,6}. Particular distribution patterns have, in addition, been related to palaeoenvironmental factors such as salinity changes and evaporative processes⁷. It was only in 1973

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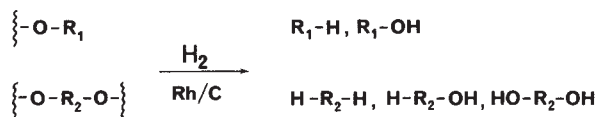


Fig. 1 Diagram showing catalytic hydrogenolysis and its products.

that Förster *et al.*⁸ were able to isolate a bacteriohopanetetrol. Rohmer and Ourisson⁹ recognized that such a highly oxygenated compound was the potential precursor of the fossil hopanes. Furthermore, its widespread occurrence in bacteria has led to the general assumption that the tetrol serves as a structural equivalent of sterols in membranes of prokaryotic organisms^{10,11}.

Despite the fact that bacteriohopanetetrol is ubiquitous in bacteria, there have been only a few reports of its presence in geological samples. Rohmer *et al.*¹² observed the coexistence of geohopanooids (hydrocarbons) and their oxygenated precursors, bacteriohopanetetrol and diplopterol, in a Recent mud. The analysis of cyanobacterial mats indicated the presence of a free tetrol¹³, and considerable amounts of bacteriohopanetetrol were found in an organic rich soil¹⁴. However free bacteriohopanetetrol has not been isolated from any pre-Quaternary sample. Because of the presence of the four hydroxy groups, it is highly unlikely that the compound survives oxidative or reductive processes in its free form during diagenesis. Rohmer *et al.*¹² could not detect bacteriohopanetetrol in the soluble low molecular weight fraction of the Messel oil shale, which is known to have undergone only very mild diagenesis.

Our work on the chemical degradation of high molecular weight organic material in sediments (that is, kerogen) has shown that molecular entities of microbial membranes can be incorporated into the matrix of the polymer and thus survive early diagenetic reactions^{15,16}. Even labile oxygenated compounds such as lignin-derived phenols could be obtained from kerogen by chemical degradation without loss or destruction of the oxygen containing functional groups¹⁷. We present evidence that intact bacteriohopanetetrol, incorporated into the polymeric matrix of an oil shale kerogen, can be released by chemical degradation.

The kerogen of the Messel oil shale was degraded by hydrogenolysis using rhodium-on-charcoal as a catalyst. A soluble organic fraction was obtained from the kerogen (organic carbon content = 69%), which was prepared by conventional HCl/HF techniques. This fraction was further separated by chromatography. Among the hydrocarbons, the pentacyclic triterpanes of the hopane type gave the most interesting results. The extractable fraction of the Messel oil shale is known to contain hopanes with carbon numbers not exceeding C₃₂ compounds¹⁸. However degradation of the kerogen revealed extended 17 β (H), 21 β (H)-

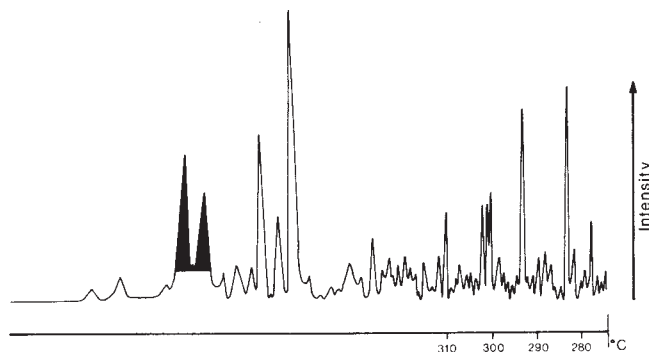


Fig. 2 Gas chromatogram of the polyalcohol fraction (acetates) of the Messel oil shale kerogen hydrogenolysis. The mass spectrum of the earlier eluting of the shaded peaks is shown in Fig. 3. Analytical conditions: 8 m $\times$ 0.25 mm SE 54 fused silica capillary column; temperature programme: 200–310 °C, 10 °C per min; carrier gas: H₂.

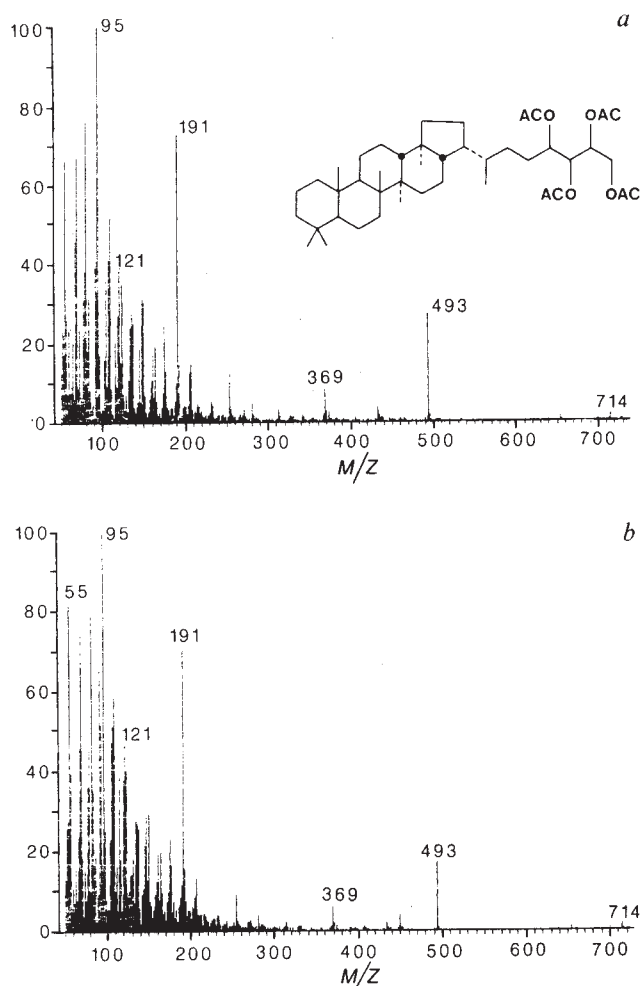


Fig. 3 Mass spectra of bacteriohopanetetrol tetraacetate from *Bacillus acidocaldarius* (a) and obtained from chemical degradation of the Messel oil shale kerogen (b). Gas chromatography-mass spectrometry, full scan mode, Varian CH7A, ionizing energy 70 eV, source temperature 250 °C; gas chromatography conditions: 30 m $\times$ 0.2 mm DB 5 fused silica capillary column; 80 °C, 5 min isothermal; 80–320 °C, 15 °C/min.

hopanes up to C₃₅. The use of deuterium instead of hydrogen during the degradation experiment gave a series of hopanes with deuterium incorporation into the side chain. On this basis, it was suggested that the hopanes were originally linked via the side chain to the polymer. It was, therefore, highly probable that bacteriohopanetetrol, linked in such a way, was the precursor of the liberated hopanes¹⁹.

Catalytic hydrogenolysis is known to cleave ether bonds to give the corresponding alcohols and hydrocarbons (Fig. 1). Ether linked biohopanooids should, thus, give free bacteriohopanetetrol in the alcohol fraction of the kerogen degradation products. In order to avoid secondary reactions, the hydrogenolysis products were acetylated (Py/Ac₂O) immediately after degradation of the kerogen. Several chromatographic steps were performed to isolate the acetylated polyalcohol fraction. Thin layer chromatography (2 mm SiO₂; hexane/ethyl acetate; 3:7 v/v) of the crude acetylated reaction product yielded a fraction with *R_F* values higher than 0.7. Thin layer chromatography was applied further to this fraction (0.5 mm SiO₂; methylene chloride). The fraction having an *R_F* value <0.15 was finally purified (0.25 mm SiO₂, thin layer chromatography; hexane/ethyl acetate; 10:7 v/v); the acetates of polyhydroxy compounds (*R_F* = 0.5) were eluted (chloroform/methanol; 1:1 v/v) and analysed by gas chromatography-mass spectrometry.

A partial gas chromatogram of the acetylated alcohols is presented in Fig. 2. Both marked peaks showed identical mass spectra which compare well with the peracetylated bacteriohopanetetrol obtained from *Bacillus acidocaldarius* (Fig. 3). Co-injection on capillary gas chromatography (GC) columns revealed the first eluting peak to have an identical retention time to the biological reference compound with the assigned stereochemistry 22(R)-17 β (H),21 β (H). The later eluting peak did not co-elute with the corresponding 22(S) isomer. This was confirmed by coinjection with reference compounds from *Acetobacter aceti* sp. *xylinum* which is the only bacterium known at present to contain both the 22(R)- and 22(S)-bacteriohopanetetrols⁹. The 22(S)-isomer elutes first on conventional capillary GC columns.

The discovery of bacteriohopanetetrol in the reaction product of a kerogen hydrogenolysate suggests the existence of intact biological membrane remnants in the high molecular weight fraction of a 50 million yr old oil shale. These membranes are obviously of prokaryotic origin and reflect a significant contribution of such microorganisms to the organic matter of the sediment. In addition the drastic kerogen isolation steps (HCl/HF treatment) have left these membrane constituents obviously unchanged. Future work will show if sediments with a more severe temperature history than the Messel oil shale contain these ether linked bacteriohopanetetrols which can be released by specific degradation reactions. Hydrogenolysis using Rh/C as a catalyst proves its powerful degradative potential by releas-

ing a variety of highly functionalized compounds in structural studies of geological polymeric organic matter.

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1. Ourisson, G., Albrecht, P. & Rohmer, M. *Scient. Am.* **25**, 44-51 (1984).
2. Van Dorsselaer, A. et al. *Tetrahedron Lett.* 1349-1352 (1974).
3. Hills, I. R. & Whitehead, E. V. *Nature* **209**, 977-978 (1966).
4. Seifert, W. & Moldovan, J. M. *Geochim. cosmochim. Acta* **42**, 473-484 (1978).
5. Mackenzie, A. S., Patience, R. L., Maxwell, J. R., Vandenbroucke, M. & Durand, B. *Geochim. cosmochim. Acta* **44**, 1709-1721 (1980).
6. Dastillung, M. & Albrecht, P. *Bull. mar. Pollut.* **7**, 13-15 (1976).
7. Ten Haven, H. L., De Leeuw, J. W. & Schenck, P. A. *Geochim. cosmochim. Acta* **49**, 2181-2191 (1985).
8. Förster, H. J., Biemann, K., Haigh, W. G., Tattrie, N. H. & Colvin, J. R. *Biochem. J.* **135**, 133-145 (1973).
9. Rohmer, M. & Ourisson, G. *Tetrahedron Lett.* 3633-3636 (1976).
10. Ourisson, G., Albrecht, P. & Rohmer, M. *Pure appl. Chem.* **51**, 709-729 (1979).
11. Kannenberg, E., Poralla, K. & Blume, A. *Naturwissenschaften* **67**, 458-459 (1980).
12. Rohmer, M., Dastillung, M. & Ourisson, G. *Naturwissenschaften* **67**, 456-458 (1980).
13. Boon, J. J. et al. in *Advances in Organic Geochemistry. 1981* (eds Björjör, M. et al.) 207-227 (Wiley, New York, 1983).
14. Ries-Kautt, M. thesis, Univ. Strasbourg (1986).
15. Michaelis, W. & Albrecht, P. *Naturwissenschaften* **66**, 420-421 (1979).
16. Chappe, B., Albrecht, P. & Michaelis, W. *Science* **217**, 65-66 (1982).
17. Mycke, B. & Michaelis, W. *Naturwissenschaften* **73**, 731-734 (1986).
18. Kimble, B. J. et al. *Geochim. cosmochim. Acta* **38**, 1165-1181 (1974).
19. Mycke, B. & Michaelis, W. *Org. Geochem.* **10**, 847-858 (1986).

A Burgess shale-like fauna from the Lower Cambrian of North Greenland

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The 'Cambrian explosion' refers to the major adaptive radiation of the metazoans during the earliest Phanerozoic, an event that is best known by the abrupt appearance of hard parts near to the Precambrian-Cambrian boundary (~550 Myr BP)¹. Deciphering this important evolutionary episode depends largely on the study of these skeletal remains^{2,3}, together with trace fossils that include burrows and trackways^{4,5}. Many of these latter fossils evidently represent the activities of soft-bodied organisms, such as worms and lightly sclerotized arthropods, whose exact identity is seldom known, not least because they can be fossilized only in exceptional circumstances⁶. However, documenting the soft-bodied representatives of early metazoan communities cannot be neglected if they accounted for a substantial fraction of these early biotas⁷. Here we report a new soft-bodied fauna from the Lower Cambrian of North Greenland. This assemblage has highly significant similarities with the younger Burgess Shale, and provides additional evidence for the geological longevity of this faunal type and its widespread distribution in deeper waters of Cambrian oceans.

The Buen Formation, from which this new assemblage derives, is a widespread unit outcropping across much of North Greenland. In Peary Land this unit is a siliciclastic shelf sequence^{8,9}. A lower sandstone unit records inshore, tidal and high-energy storm-dominated environments, whereas the overlying recessive unit of predominantly mudstones reflects a low-energy, deeper outer-shelf environment deposited close to storm-wave base¹⁰. The new assemblage is represented by a small collection from

a single locality near J. P. Koch Fjord, western Peary Land (Fig. 1), where a small fault-bounded block exposes fissile black shales near the top of the formation.

The Buen Formation is dated as mid-to-late early Cambrian, and the lower part of the overlying Brønlund Fjord Group also yields late Lower Cambrian faunas^{9,11}. In terms of north American Lower Cambrian biostratigraphy¹² a position within the lower part of the *Bonnia-Olenellus* Zone is most likely, but an even older age within the underlying *Nevadella* Zone is conceivable¹¹. The occurrence in the assemblage of an olenellacean trilobite broadly supports the postulated age, but the shelly fauna lacks other trilobites and is restricted otherwise to

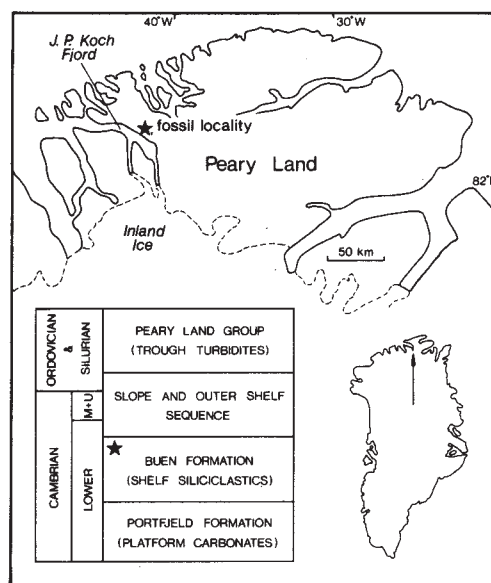


Fig. 1 Locality of new soft-bodied fauna in Peary Land, northern Greenland (and arrow on map lower right inset). Inset column (lower left) shows position of Buen Formation in the regional stratigraphy. The fauna occurs near the top of the Buen Formation, in beds equivalent to either the *Nevadella* or *Bonnia-Olenellus* zones (ref. 11).

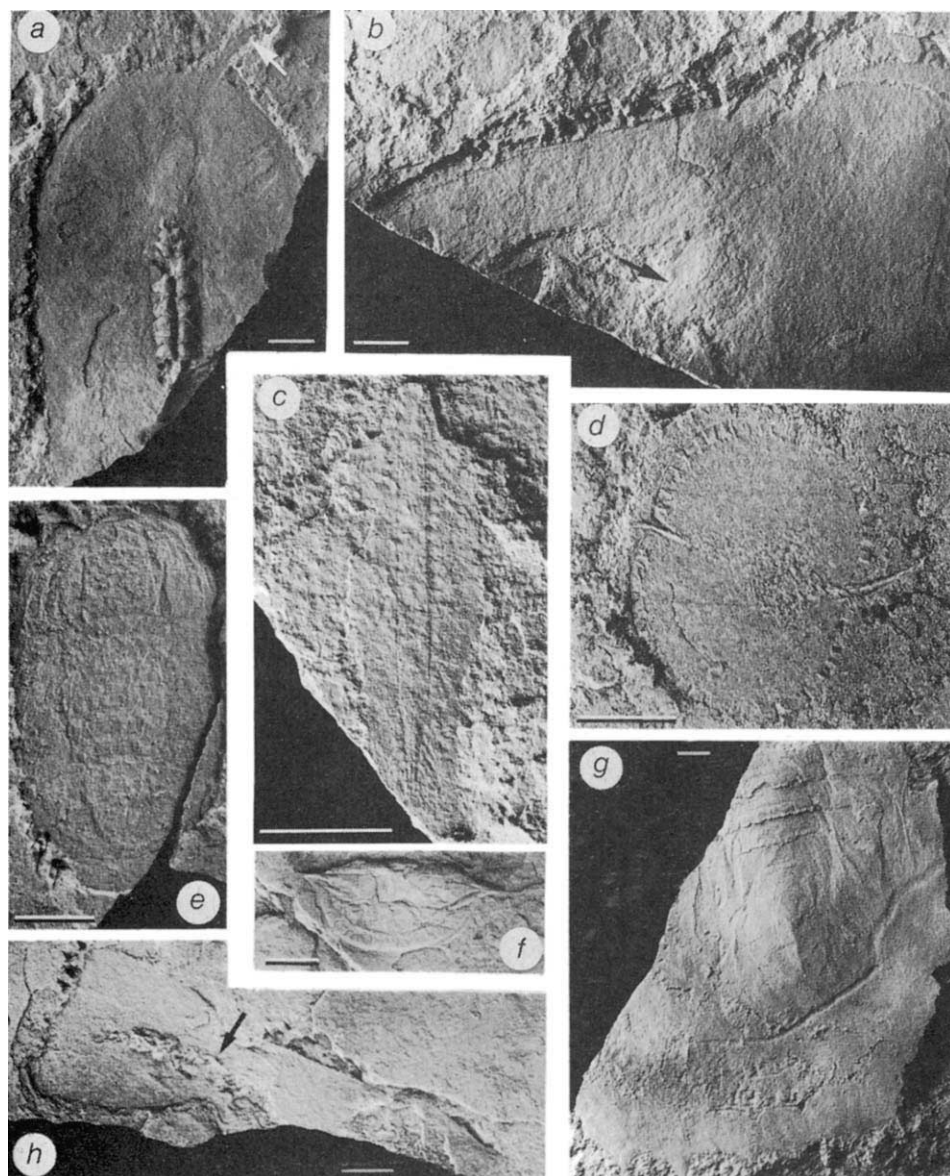


Fig. 2 Arthropods from the Buen Formation, Peary Land, northern Greenland. *a*, Anterior half showing oval headshield and segmented thorax. Note anterior antennae (arrowed) and prominent median gut-fill (Geological Museum, Copenhagen specimen no. MGUH 17512). *b*, Incomplete specimen with bivalved carapace, protruding segmented abdomen, and possible appendages (arrowed) (MGUH 17513). *c*, Posterior section of *Leanchoilila*-like arthropod with segmented thorax and spinose telson (MGUH 17514). *d*, Bradoriid carapace with prominent spines (MGUH 17515). *e*, Isopygous form with cephalon and posterior shield separated by six thoracic segments (MGUH 17517). *f*, Carapace of *Isoxys* (MGUH 17518). *g*, Posterior of large arthropod with marginal spines (MGUH 17516). *h*, Posterior section showing abdomen terminating in a tail fan, with gut contents (arrowed) (MGUH 17519). All specimens from Geological Survey of Greenland sample 319571, deposited in the type collection, Geological Museum, Copenhagen (MGUH). Scale bars, 5 mm.

inarticulate brachiopods. Siliceous sponge spicules are very abundant, and articulated material of the demosponge *Choia*¹³ is consistent with the conditions of exceptional preservation documented here.

The soft-bodied component of this locality is dominated by non-trilobitic arthropods that lack mineralized skeletons. The most abundant form (Fig. 2*d*) is represented by a semi-circular bivalved carapace (7.5–14 mm long), with short anterior and posterior spines projecting upwards from the hinge line at a steep angle. This arthropod appears to be most similar to Lower Cambrian bradoriids such as *Sunella*^{14,15}, but it differs in being larger and having spines of equal length. Somewhat similar to this presumed bradoriid is the larger *Isoxys* (Fig. 2*f*), with longer anterior and posterior spines that project without pronounced curvature.

The largest of these arthropods, growing to at least 130 mm long, is represented by 10 specimens. The smooth cephalon was semi-circular in outline, and bore a pair of elongate segmented antennae that arose adjacent to a poorly defined structure that appears to represent a ventral plate or hypostome (Fig. 2*a*). The thoracic region was divided into approximately 12–13 narrow tergites, and the posterior shield seems to have been broadly similar to the anterior cephalon. Incomplete specimens, questionably assigned to this form, show a posterior shield with at

least four pairs of marginal spines (Fig. 2*g*). Information on the internal anatomy is restricted to a gut trace, that includes a prominent nodular infill (Fig. 2*a*) with impressed striations that may represent enteric musculature. Overall it appears to be most similar to *Tegopelte*, an aberrant trilobite from the Burgess Shale that grew to over 250 mm in length¹⁶. One obvious difference is that *Tegopelte* had three thoracic tergites, but these probably represent a fused complement of at least 10 somites.

The remaining arthropods represent at least three taxa, but are all rare. One type has roughly equal-sized (isopygous) cephalon and posterior shield that are separated by six thoracic tergites (Fig. 2*e*). An axial region defines a longitudinal trilobation along much of the body. This arthropod has a generalized resemblance to a number of Cambrian forms; for example, *Livia plana* from the Lower Cambrian of northern Poland¹⁷. Another distinctive arthropod bore a semi-circular bivalved carapace, from which appendages apparently protruded (Fig. 2*b*). Poor preservation precludes confident identification, but they may have included filamentous gills and possibly ambulatory appendages. Projecting from the carapace was a relatively slender abdomen, composed of at least six somites, and lacking appendages. Similarities may exist with such Burgess shale arthropods as *Canadaspis perfecta*¹⁸ and *Waptia fieldensis*^{19,20}. An incomplete specimen, possibly distinct from the bivalved arthropod,

has a prominent tail fan composed of a telson flanked by flattened plates (Fig. 2h). A prominent gut trace shows that the anus was located on the telson. Another fragmentary specimen is represented by a series of about 20 thoracic segments, that terminate in an elongate telson bearing a prominent series of at least 8 spines on either side (Fig. 2c). A spiny telson is particularly characteristic of the arthropod *Leanchoilia*, best known from the Burgess Shale²¹, as well as other middle Cambrian horizons^{22,23}.

This new soft-bodied fauna is important for several reasons. First, it increases our knowledge of Cambrian diversity and confirms the importance of arthropods, especially lightly sclerotized taxa. In this assemblage, and better known examples such as the middle Cambrian Burgess Shale, these arthropods far outnumbered the better-known trilobites in terms of both numbers of individuals and biomass⁷.

Second, in general terms this fauna has striking similarities with a number of soft-bodied Cambrian biotas such as the well-known Burgess Shale^{7,24,25}, immediately adjacent localities²² and additional horizons from the United States^{23,26-31} and China³²⁻³⁴. Together, they reveal a broadly comparable Cambrian biota with a distinct identity. These exceptional faunas share the significant character of having been deposited in deeper water shales, on the margin of the outer detrital belt (or its equivalent) and facing the open ocean. This soft-bodied fauna in north Greenland adds to our knowledge of the circum-Laurentian distribution of Burgess Shale-like faunas³⁵. Moreover, similarities between the soft-bodied fauna of South China³²⁻³⁴ and those encircling the Laurentian craton, suggest that migration by the ocean floor across a 'proto-Pacific' was possible, in a manner comparable to that inferred for Upper Cambrian deep-water trilobites³⁶.

Third, there is evidence that a number of the Burgess Shale-type genera had unusually long stratigraphic durations. This gives these soft-bodied assemblages a rather conservative aspect in evolutionary terms, and finds a significant parallel in the generic longevity of other deep-water biotas³⁷. The occurrence reported here is a particularly important addition, because it and the Chinese³²⁻³⁴ occurrences are amongst the earliest known. The extension of stratigraphic ranges of at least some Burgess Shale-like taxa back into the early Cambrian also suggests that they were an integral part of the initial diversification of metazoans.

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- Conway Morris, S. *Am. Scient.* (in the press).
- Bengtson, S. *Acta Univ. Upsalienensis* 415, 1-71 (1977).
- Glaessner, M. F. *The Dawn of Animal Life, a Biohistorical Study* (Cambridge University Press, 1984).
- Alpert, S. P. *Geol. J. Spec. Issue* 9, 1-8 (1977).
- Crimes, T. P. & Anderson, M. M. *J. Paleont.* 59, 310-343 (1985).
- Whittington, H. B. & Conway Morris, S. (eds). *Phil. Trans. R. Soc. B311*, 1-192 (1985).
- Conway Morris, S. *Palaeontology* 29, 423-467 (1986).
- Jepsen, H. F. *Bull. Grøn. geol. Unders.* 96, 1-42 (1971).
- Peel, J. S. *Can. Soc. Petrol. geol. Mem.* 8, 309-330 (1982).
- Higgins, A. K., Ineson, J. R., Peel, J. S., Surlyk, F. & Sønderholm, M. in *The Geology of North America Vol. E* (ed. Trettin, H. P.) (Geological Survey of Canada, Ottawa, in the press).
- Palmer, A. R. & Peel, J. S. *Rapp. Grøn. geol. Unders.* 91, 29-36 (1979).
- Fritz, W. H. *Bull. geol. Surv. Can.* 212, 1-90 (1972).
- Rigby, J. K., *Rapp. Grøn. geol. Unders.* (in the press).
- Huo Shicheng *Acta palaeont. sin.* 4, 425-445 (1956).
- Huo Shicheng & Shu Degan *Cambrian Bradorids of South China* (Northwest University Publishing House, Xi'an, 1985).
- Whittington, H. B. *J. Paleont.* 59, 1251-1274 (1985).
- Lendzion, K. *Kwart. geol.* 19, 237-242 (1975).
- Briggs, D. E. G. *Phil. Trans. R. Soc. B281*, 439-487 (1978).
- Walcott, C. D. *Smithson. misc. Colls* 57, 145-228 (1912).
- Hughes, C. P. in *Atlas of the Burgess Shale* (ed. Conway Morris, S.) 1-31 (Palaeontological Association, London, 1982).
- Bruton, D. L. & Whittington, H. B. *Phil. Trans. R. Soc. B300*, 553-585 (1983).

- Collins, D. *et al. Science* 222, 163-167 (1983).
- Briggs, D. E. G. & Robison, R. A. *Paleont. Contrib. Univ. Kansas* 111, 1-23 (1984).
- Conway Morris, S. *Ann. Rev. Ecol. Syst.* 10, 327-349 (1979).
- Whittington, H. B. *The Burgess Shale* (Yale University Press, New Haven, 1985).
- Resser, C. E. & Howell, B. F. *Bull. geol. Soc. Am.* 49, 195-248 (1938).
- Campbell, L. & Kaufman, M. E. *Proc. Pa. Acad. Sci.* 43, 172-176 (1969).
- Robison, R. A. & Richards, B. C. *Paleont. Contr. Univ. Kans* 106, 1-19 (1981).
- Robison, R. A. *Paleont. Contr. Univ. Kans* 112, 1-8 (1984).
- Robison, R. A. *J. Paleont.* 59, 226-235 (1985).
- Conway Morris, S. & Robison, R. A. *Paleont. Contr. Univ. Kans* 117, 1-22 (1986).
- P'an Kiang *Acta palaeont. sin.* 5, 523-526 (1957).
- Jiang Zhiwen in *The Sinian-Cambrian boundary in eastern Yunnan, China* (eds Luo Huilin, Jiang Zhiwen, Wu Xiche, Song Xuiliang, Ouyang Lin *et al.*) 215 (People's Publishing House, Yunnan, 1982).
- Zhang Wen-tang & Hou Xian-guang *Acta palaeont. sin.* 24, 591-595 (1985).
- Conway Morris, S. *Phil. Trans. R. Soc. B311*, 49-65 (1985).
- Taylor, M. E. & Cook, H. E. *Geology Stud. Brigham Young Univ.* 23, 181-214 (1976).
- Fortey, R. A. *Paleobiology* 6, 24-31 (1980).

Ketamine-xylazine anaesthesia blocks consolidation of ocular dominance changes in kitten visual cortex

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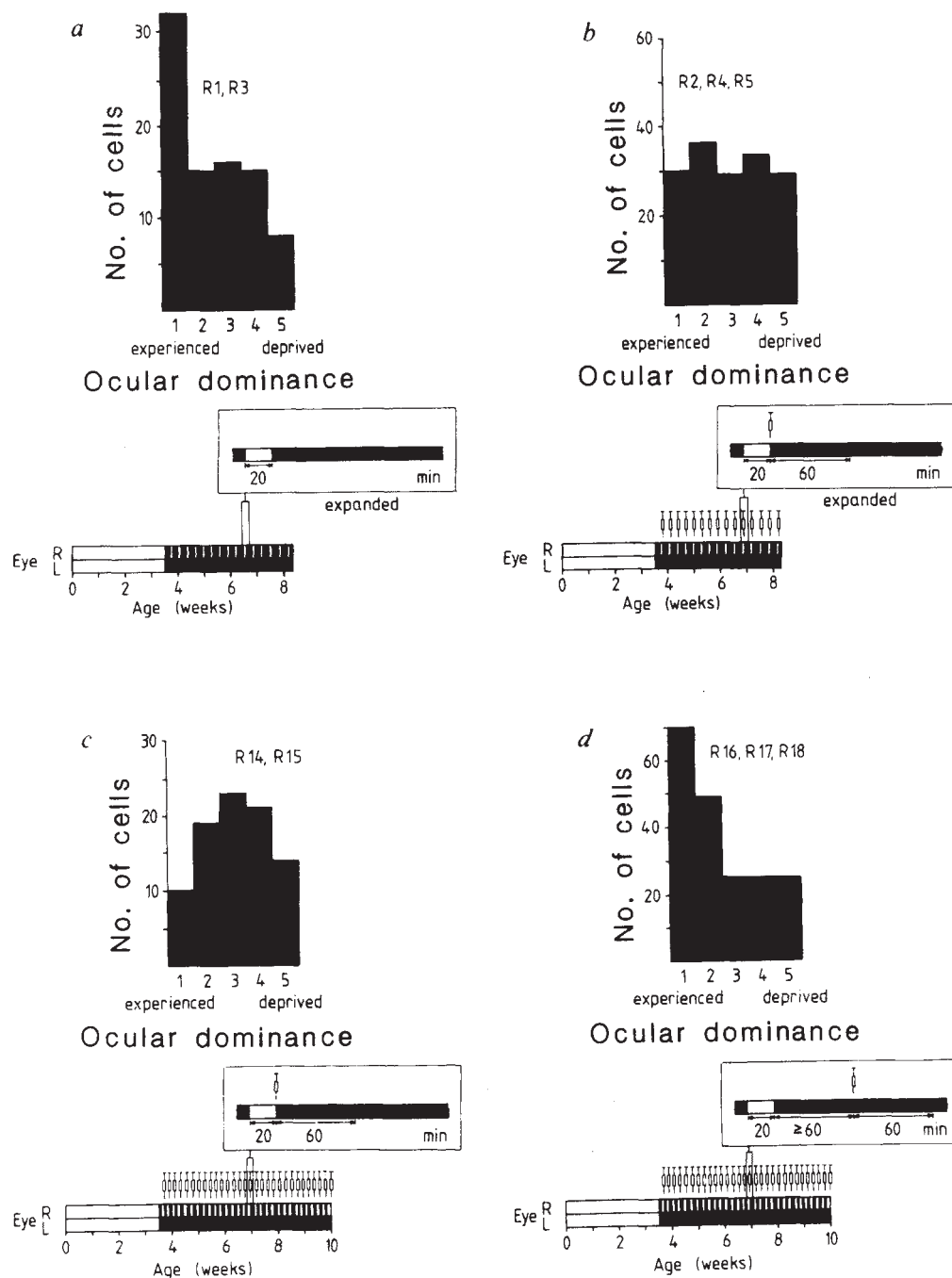
In the visual cortex of mammals, response properties of single neurons can be changed by restricted visual experience during early postnatal development¹. Covering one eye for four to eight hours when kittens are at the peak of the sensitive period is sufficient to weaken the influence of the occluded eye on cortical neurons resulting in a noticeable shift of ocular dominance towards the open eye²⁻⁵. The underlying changes in synaptic connections do not occur so readily when a kitten is anaesthetized and paralysed⁶⁻⁸. We report here that an ocular dominance shift is prevented in alert kittens that receive repeated brief monocular exposures when these are followed by ketamine-xylazine anaesthesia. This retrograde effect on cortical plasticity suggests that the process by which synaptic activity is converted into structural changes has been disturbed.

Kittens were kept in a dark room from 3.5 weeks of age. On every other day they were exposed to a high-contrast visual environment for 20 min with one eye occluded by a black contact lens. This was repeated 15 times, resulting in 5 h total monocular experience. After each 20-min exposure the kittens were returned to the dark. Control animals were given sham injections of NaCl solution (0.9%, 2 ml per kg body weight). The experimental animals received an intramuscular injection of ketamine-hydrochloride (Ketanest 1%, 25 mg kg⁻¹) supplemented with xylazine (Rompun 5%, 2 mg kg⁻¹). Ketamine-xylazine is a standard veterinary combination anaesthesia used for its rapid onset and minimal effects on blood pressure. With our dosage anaesthesia lasted for about one hour (mean 74 ± 12 min). All kittens were weighed regularly, and no difference in their rate of physical development was seen from their body weight.

Two days after the last exposure session a double-blind recording experiment in striate cortex was performed. Standard physiological techniques were used^{9,10}. Animals were anaesthetized with halothane (0.4%) and N₂O/O₂ (70%:30%). Individual results are shown in Table 1. The ocular dominance (OD) distributions of two control (R1, R3) and three experimental animals (R2, R4, R5) are shown in Fig. 1a and b, respectively. Kittens R1 and R3 showed a clear dominance of the exposed eye, but the three kittens with regular post-exposure anaesthesia had an even OD distribution.

To ensure that the total exposure time of 5 h had not been too short, we performed another series of experiments in which we gave 30 exposures of 20 min duration to one eye on every day, the total exposure time amounting to 10 h. Again, recording

Fig. 1 The ocular dominance distributions of single neurons in visual cortex (area 17) of both cerebral hemispheres from four different groups of kittens. The ocular dominance (OD) of a cell indicates the relative degree of excitability from the two eyes by visual stimulation and is grouped into five categories: class 1(5), only driven through right (left) eye; class 2(4), larger response when stimulated through right (left) eye; class 3, about equally driven through either eye. OD ratings were first determined by hand and were then confirmed quantitatively from PSTHs averaged in at least four, usually eight directions of a moving light bar. Below each histogram the experimental history of the group is shown. Open and filled blocks correspond to light and dark rearing respectively. Three groups of animals (*b, c, d*) were given intramuscular injections of ketamine-xylazine after each visual exposure as indicated by the syringe symbols. The precise time of anaesthesia is shown in the inserts on an expanded time scale. All receptive fields were within the central 10° of the visual field. No difference was found between data collected from the two cerebral hemispheres. *a*, Control kittens with 5 h total monocular experience given for 20 min every other day, 15 times. Animals were alert and lightly restrained during exposure for continuous stimulation in a high-contrast visual environment of 170 cd m⁻² mean luminance. *b*, Same exposure protocol as in *a*, but here the kittens were anaesthetized immediately after each exposure. Ocular dominance was not shifted in these animals. *c*, Same protocol as *b*, but total monocular exposure was extended to 10 h. Exposure was given every day. *d*, Same protocol as *c*, but kittens were given a break of one (R16) or six hours (R17, R18) in total darkness after each exposure session before receiving anaesthesia. The shift of ocular dominance in these kittens indicates that retrograde duration of impairment is probably <1 h.



was done blind and all ratings were confirmed quantitatively from peri-stimulus-time histograms (PSTHs). Two kittens (R14 and R15) received the anaesthetic immediately after each exposure. Again their OD distribution showed no signs of a shift towards the exposed eye (Fig. 1c).

As further controls, three kittens (R16, R17, R18) with the same exposure schedule received the anaesthetic only after a delay of 1 or 6 h spent in the dark. All three animals showed an OD shift in striate cortex (Fig. 1d). This result demonstrates most clearly that anaesthesia did not have an effect on the physical state or on the rate of development of the animals. Moreover, it excludes an anterograde effect of anaesthesia and shows that the retrograde impairment of binocular plasticity lasts not more than an hour. This can also be demonstrated by extending the duration of exposure sessions to one hour (Fig. 2). If in this case anaesthesia is given immediately after each exposure, an OD shift is not prevented (Fig. 2a). The shift is

as clear as in two control cats that received only sham injections of NaCl (Fig. 2b).

The results of this study show that a short period exists during which ketamine-xylazine anaesthesia can retrogradely interfere with the plasticity of binocular connections in the visual cortex of young kittens. The synaptic changes that are normally triggered by neuronal activity^{9,11} are prevented or disrupted. The period is reminiscent of what has been termed a 'consolidation period' by others¹². Our results indicate, however, that consolidation occurs much more rapidly than had been assumed in other studies, which tested the effects of delay after a single 24-h monocular exposure^{3,4}. Clearly, in these experiments initial consolidation had already taken place during exposure.

It is of particular interest whether the retrograde impairment of cortical plasticity is specific for ketamine-xylazine. Preliminary experiments in two kittens (U. Egert and J.P.R., in preparation) indicate that the effect cannot be induced by

Fig. 2 Comparisons of ocular dominance distributions from (a) two kittens that were anaesthetized with ketamine-xylazine immediately after each of ten monocular exposures with those from (b) two control animals that received only sham injections of saline. In all cases exposure lasted for 60 min instead of 20 min as in Fig. 1. All animals show a shift of ocular dominance, which supports the conclusion that the duration of a 'consolidation period' is <1 h. Columns marked 'u' show visually unresponsive neurons.

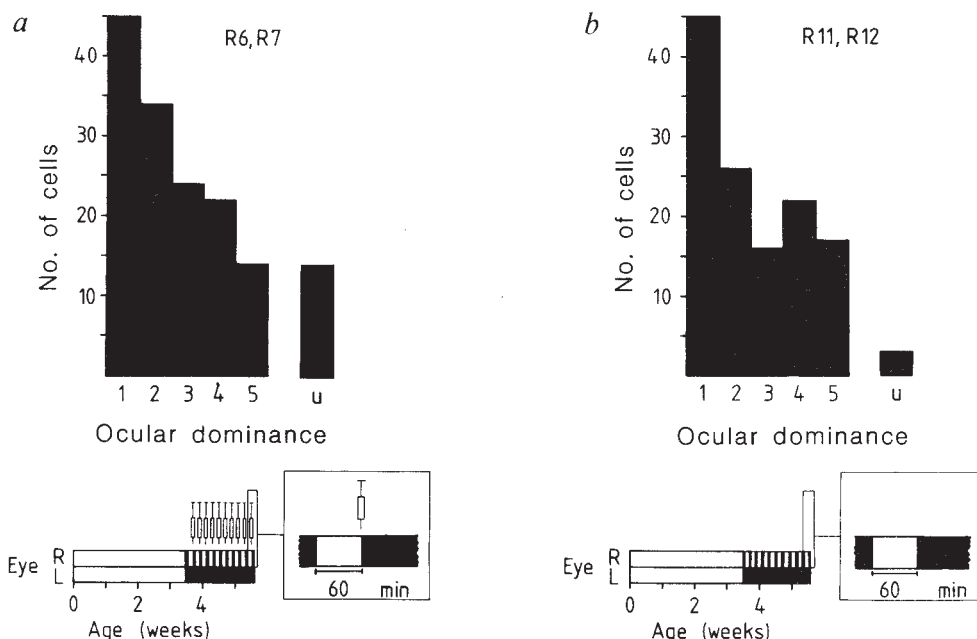


Table 1 Individual results of single unit recording from striate cortex in 14 kittens

Group no.	Animal no.	No. of units analysed	No. of unresponsive units	No. of OD determined in hemisphere		No. of units in each ocular dominance group					Weighted OD index ($I = \frac{n_1 + 0.5n_2}{n_5 + 0.5n_4}$)
				contra	ipsi to deprived eye	n_1	n_2	n_3	n_4	n_5	
1a	R1	51	15	30	6	13	7	7	5	4	2.5
	R3	70	20	25	25	19	8	9	10	4	2.6
1b	R2	57	17	20	20	8	9	4	8	11	0.8
	R4	65	9	34	22	8	14	15	14	5	1.25
1c	R5	83	20	25	36	14	13	10	11	13	1.1
	R14	29	6	22	1	4	2	7	5	5	0.7
1d	R15	82	16	31	33	6	17	16	16	9	0.9
	R16	74	11	29	30	28	17	7	4	3	7.3
2a	R17	81	7	35	36	23	16	10	12	10	1.9
	R18	72	7	24	40	19	16	8	9	12	1.6
2b	R6	75	7	33	34	17	18	11	13	8	1.8
	R7	80	7	35	37	28	16	13	9	6	3.4
2b	R11	69	1	35	33	24	13	8	12	11	1.8
	R12	61	2	30	28	21	13	8	10	6	2.5

The group numbers correspond to the Figure in which data and experimental protocol are shown. The non-parametric Mann-Whitney u -test was used to compare the experimental and control groups. If the weighted OD indices of the animals in control groups 1a and 1d are tested against the animals in experimental groups 1b and 1c, a significant difference of $P < 0.005$ is obtained. Comparison of 2a/2b with 1b/1c yields $P < 0.01$.

xylazine alone, which is an α -adrenoceptor agonist^{13,14}. Ketamine, on the other hand, is known to interfere with memory processes, and its psychotomimetic effects have long been known^{15,16}. Recently it has been found to be a blocker of NMDA-receptors for glutamate^{15,17,18}, blockage of which prevents LTP in hippocampal slices^{19,20} and spatial discrimination learning²¹.

Clearly, further experiments are needed to determine the pharmacological mechanisms of synaptic consolidation. The important aspect of the present findings is that they demonstrate the existence of a consolidation period for visual cortical plasticity. Consolidation effects are well-established in the psychology of learning^{22,23} and our results suggest that the same processes may be involved in visual plasticity as in long-term memory.

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1. Wiesel, T. N. *Nature* **299**, 583-592 (1982).

- Peck, C. K. & Blakemore, C. *Expl Brain Res.* **22**, 57-68 (1975).
- Freeman, R. D. & Olson, C. R. *Nature* **282**, 404-406 (1979).
- Freeman, R. D. & Olson, C. R. *J. Neurophysiol.* **47**, 139-150 (1982).
- Movshon, J. A. & Duersteler, M. R. *J. Neurophysiol.* **40**, 1255-1265 (1977).
- Buisseret, P., Gary-Bobo, E. & Imbert, M. *Nature* **272**, 816-817 (1978).
- Freeman, R. D. & Bonds, A. B. *Science* **206**, 1093-1095 (1979).
- Singer, W. & Rauschecker, J. P. *Expl Brain Res.* **47**, 223-233 (1982).
- Rauschecker, J. P. & Singer, W. *J. Physiol., Lond.* **310**, 215-239 (1981).
- Rauschecker, J. P. & Harris, L. R. *Expl Brain Res.* **50**, 69-83 (1983).
- Rauschecker, J. P. & Singer, W. *Nature* **280**, 58-60 (1979).
- Pettigrew, J. D. & Garey, L. J. *Brain Res.* **66**, 160-164 (1974).
- MacDonald, A. & McGrath, J. C. *Br. J. Pharmac.* **71**, 445-458 (1980).
- Docherty, J. R. & Starke, K. *Br. J. Pharmac.* **76**, 327-335 (1982).
- Thomson, A. M., West, D. C. & Lodge, D. *Nature* **313**, 479-481 (1985).
- Lodge, D. & Johnston, G. A. R. *Neurosci. Lett.* **56**, 371-375 (1985).
- Anis, N. A., Burton, N. R., Berry, S. C. & Lodge, D. *Br. J. Pharmac.* **79**, 565-575 (1983).
- Watkins, J. C. & Evans, R. H. A. *Rev. Pharmac. Tox.* **21**, 165-204 (1981).
- Collingridge, G. L., Kehl, S. J. & McLennan, H. J. *J. Physiol., Lond.* **334**, 19-31 (1983).
- Harris, E. W., Ganong, A. H. & Cotman, C. W. *Brain Res.* **323**, 132-137 (1984).
- Morris, R. G. M., Anderson, E., Lynch, G. S. & Baudry, M. *Nature* **319**, 774-776 (1986).
- McGaugh, J. L. & Herz, M. J. *Memory Consolidation* (Albion, San Francisco 1972).
- Squire, L. R. *Science* **232**, 1612-1619 (1986).

Long-distance intraretinal connections in birds

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Electrophysiological experiments have shown in both birds and mammals that remote parts of the retina, several millimetres apart, interact at the retinal level^{1,2}. The anatomical basis of this is poorly understood, although in mammals some cells in the ganglion cell layer have axons that terminate in the inner plexiform layer several millimetres from the cell body^{3,4}. In birds, the longest previously reported intraretinal connections were from amacrine cells, extending only a few hundred microns⁵. But we here describe very long connections that span almost the entire extent of the retina in chicks and chick embryos. The parent cell bodies are in the inner nuclear layer of the ventral half of the retina, and they project in topographical order onto the dorsal half. They do not project to the brain. They may be involved in selective switching of attention between the upper and lower parts of the visual field, at an unprecedentedly early stage of visual processing.

We have demonstrated this projection by retrograde transport of the intensely fluorescent carbocyanine dye 1'-di-octadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), which is known to be transported both anterogradely and retrogradely^{6,7}. The dye was inserted into the retinas of anaesthetized embryos and newly hatched chicks (see Fig. 1 legend). It hardly diffused at all, as is shown by the fact that the only labelled optic fibres were restricted to a narrow band 0.1–0.5 mm wide, running through the site of dye placement to the optic nerve-head, and in the opposite direction towards the ganglion and displaced ganglion cell bodies of origin, which were labelled directly along it.

Remote from this stream of labelled cells, other retinal neurons that we shall call 'propriorectal cells' were labelled in the inner half of the inner nuclear layer. These cells were mostly of two types, equally numerous (Figs 1–3). Type I was the larger, 15–20 μm in diameter. It lay close to the inner plexiform layer and resembled Cajal's 'stratified amacrine cells of the first level'⁸, displaying several labelled dendrites which could often be traced for more than 100 μm in the most external level of the inner plexiform layer (Cajal's lamina I; ref. 8). Type II was only slightly smaller, about 15 μm in diameter. It lay deeper in the inner nuclear layer and appeared pear-shaped, with a single thick process extending from the cell body to lamina I of the inner plexiform layer where it could sometimes be seen to branch, although the dendrites could never be followed for more than a few microns; it resembled Cajal's 'association amacrine cells'⁸. From both types labelled axons ran in the outer part of

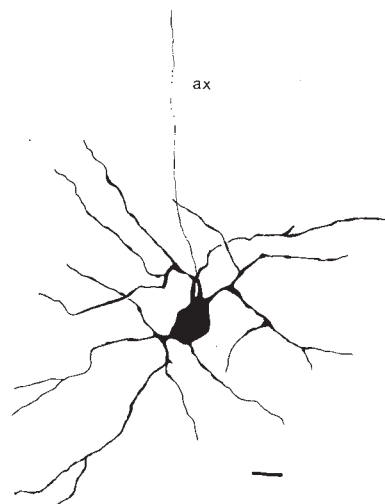


Fig. 2 Tracing of a propriorectal type I neuron in the ventro-nasal part of a retinal flat-mount from a 4-day-old chick labelled with a fleck of DiI in the dorso-nasal retina; ax, axon. Nasal is right and dorsal is up; scale bar, 10 μm .

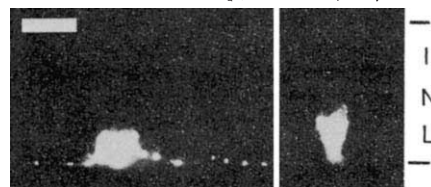


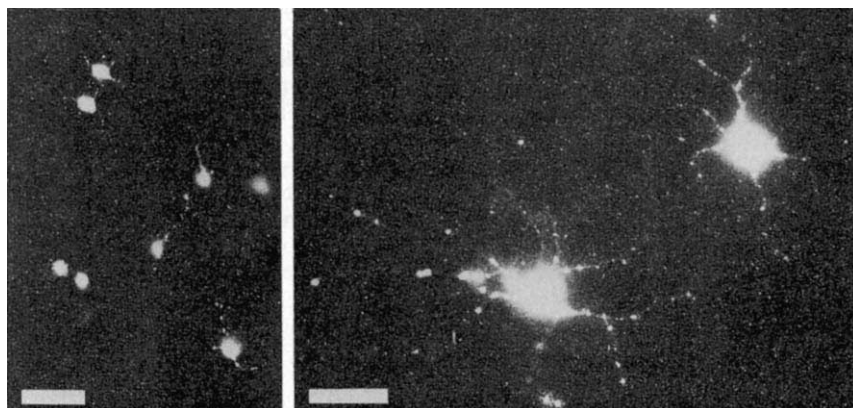
Fig. 3 Type I (left) and type II (right) propriorectal cells from a 4-day-old chick viewed in a transverse section of the retina to show the differences in their morphology, and in their depth in the inner nuclear layer. Such sections were prepared by removing flat-mounted retinas from the slide and re-sectioning them in a cryostat at 40 μm , after immersion in 30% buffered sucrose. INL, inner nuclear layer; scale bar, 20 μm .

the inner plexiform layer directly towards the dye placement site, where they could no longer be traced because of the high background. Occasionally they appeared to yield terminal arbors just as they entered the high background region.

Experiments on embryos of different ages indicated that the propriorectal projection was detectable on embryonic day 11 (E11) and was fully developed 2–3 days later.

We have studied the topography of the projection in 39 retinas, in 14 of which the distribution of labelled cells was charted quantitatively using a microscope linked to a computer. The position of the dye-insertion was systematically varied so as to test all parts of the retina except the most central region, which was inaccessible. The results are summarized in Fig. 4. The labelled propriorectal cells were found exclusively in the ventral

Fig. 1 Propriorectal cells, viewed in a retinal flat-mount from a 3-day-old chick. Left, 5 type I cells (upper and right) and 2 type II cells (lower left). Right, 2 type I cells at higher magnification. Scale bars: left, 75 μm ; right, 30 μm . In all experiments a fleck of DiI was inserted into the right retina through a small cut in the sclera and choroid. After 2–9 days, the birds were killed and the labelled eye removed. The cornea was excised and the eye immersed in 10% formalin in cacodylate buffer (0.1 M, pH 7.2) for 3 days at 4 °C. The retina was then flat-mounted, with the vitreal surface up, in an aqueous solution of 90% glycerol and 2% propyl gallate, a coverslip was applied, and the retina viewed with a fluorescent microscope using filters appropriate for rhodamine.



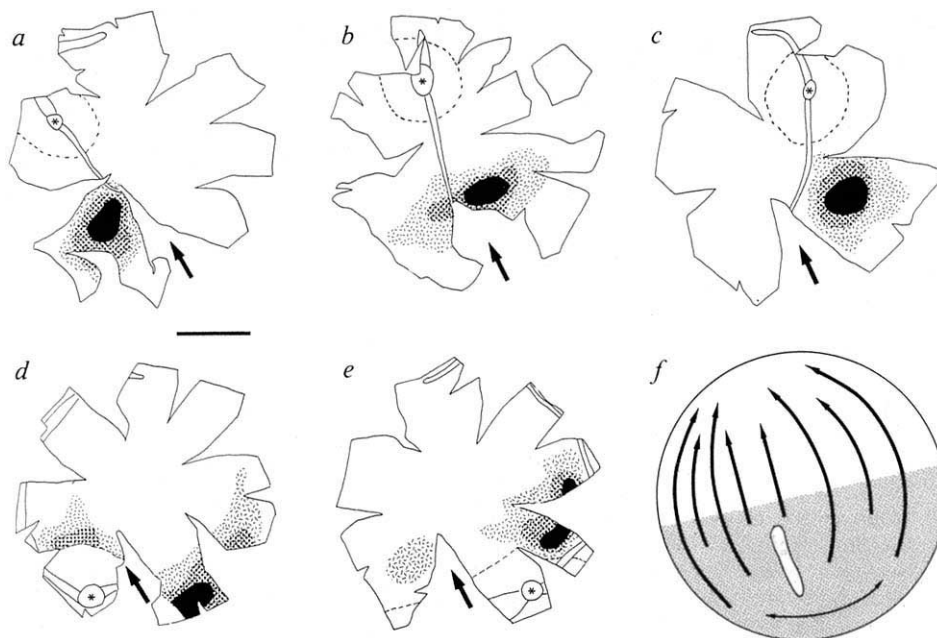


Fig. 4 The topographical arrangement of the proprioretinal projection viewed in flat-mounts. *a-e*, Distribution of the labelled neurons in five 4-day-old chicks following the placement of DiI (asterisks) in different parts of the right retina. The interrupted lines circumscribe the region immediately around the dye fleck where many cells are labelled in all layers. The double line stretching from the dye fleck towards and away from the optic nerve-head (elongated in birds—see *f*) denotes the band of labelled optic axons; the stream of labelled ganglion and displaced ganglion cells lies along this band distal to the dye fleck. Arrows, optic fissure, which allows proper orientation of the retina. The density of labelled proprioretinal cells is as shown: black, 31–80 cells mm^{-2} ; heavy stippling, 16–30 cells mm^{-2} ; light stippling, 6–15 cells per mm^{-2} . Scale bar, 5 mm. *f*, Summary of the topography. The proprioretinal cells were found exclusively in the stippled region. They project as indicated by the arrows. The cigar-shaped profile represents the optic nerve-head. In all diagrams, nasal is right and dorsal is up.

half of the retina. From here they project both to the dorsal retina and within the ventral half. The former projection shows a clear topographical organization. The ventro-nasal sector projects to the dorso-nasal, the mid-ventral to the mid-dorsal, the ventro-temporal to the dorso-temporal. In addition, the more ventral parts of the ventral hemiretina appear to project the more dorsally in the dorsal half (Fig. 4*f*). It was difficult to define the projection pattern within the ventral region, but many proprioretinal cells in each of the ventral quadrants have axons which pass beyond the optic fissure into the adjacent one (Fig. 4*d,e*).

The maximal density of labelled proprioretinal cells was typically 80 mm^{-2} and the area of the ventral hemiretina was about 90 mm^2 , which suggests there were not less than 7,000 of them in each retina.

The fact that the proprioretinal cells had labelled axons running directly to the dye fleck and not towards the optic nerve-head suggested that they did not project to the brain, but further experiments were needed to check this point. They might conceivably have had an unlabelled axonal branch running to the optic nerve-head. Or, less likely, the axon destined for the nerve-head might have followed an indirect trajectory through the site of dye placement. Both these possibilities were tested in two series of double labelling experiments using DiI and Fast Blue⁹ as complementary dyes.

For technical reasons we used embryos, but at an age when the optic and proprioretinal projections are fully developed. In one series we inserted a fleck of DiI in the dorso-temporal retina at E13–E14, injected the contralateral mid-brain and thalamus with Fast Blue (2% in water, several injections, total 1.6 μl) at E14, and killed the embryo at E16. In the other series we first injected the Fast Blue in the brain at E12, inserted the fleck of DiI in the retina at E14, and killed the embryo at E16. Nine embryos of the first series and three of the second were well labelled with both dyes.

In frozen sections through the mid-brain and thalamus there was a heavy deposit of Fast Blue in all the primary optic centres, including the ectomammillary nucleus and optic tectum. This led to most retinal ganglion cells being retrogradely labelled. In the inner nuclear layer, blue-labelled displaced ganglion cells^{10,11} and red-labelled proprioretinal cells occurred side by side, but none was double-labelled, indicating that none of the proprioretinal cells projects to the brain.

In conclusion, we have shown that long proprioretinal projections occur, and that they originate exclusively in the ventral retina, which represents the upper part of the visual field. A similar bias towards the ventral retina is shown in chicks by the centrifugal fibres from the brain (ref. 7, unpublished). We therefore speculate that the intraretinal connections may be involved in a system for switching attention between the upper and lower halves of the visual field under partial centrifugal control.

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- McIlwain, J. T. *J. Neurophysiol.* **27**, 1154–1173 (1964).
- Holden, A. L. *Vision Res.* **17**, 665–666 (1977).
- Gallego, A. & Cruz, J. *Science* **150**, 1313–1314 (1965).
- Dacey, D. M. *J. comp. Neurol.* **242**, 247–262 (1985).
- Mariani, A. P. *Nature* **298**, 654–655 (1982).
- Honig, M. G. & Hume, R. I. *J. Cell Biol.* **103**, 171–187 (1986).
- Catsicas, S., Thanos, S. & Clarke, P. G. H. *Soc. Neurosci. Abstr.* **12**, 984 (1987).
- Ramón y Cajal, S. in *The Vertebrate Retina* (by Rodieck, R. W.) 773–904 (Freeman, San Francisco, 1973).
- Bentivoglio, M., Kuypers, H. G. J. M., Catsman-Berrevoets, C. E., Loewe, H. & Dann, O. *Neurosci. Lett.* **18**, 25–30 (1980).
- Heaton, M. B., Alvarez, I. M. & Crandall, J. E. *Anat. Embryol.* **155**, 161–178 (1979).
- Reiner, A., Brecha, N. & Karten, H. J. *Neuroscience* **4**, 1679–1688 (1979).

The v-myc oncogene is sufficient to induce growth transformation of chick neuroretina cells

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A number of studies have shown that full transformation of non-established rodent fibroblasts can be efficiently achieved *in vitro* by the concerted action of two oncogenes belonging to different complementation groups (reviewed in ref. 1). Extension of the two-genes carcinogenesis model to other differentiated cell types, presumably endowed with different controls of growth, is desirable for a better understanding of questions such as the host cell selectivity of oncogene action. A recent report² claimed that cooperation between two oncogenes, v-myc and v-mil, is required to achieve transformation of chicken embryo neuroretina cells, which are characterized by a limited growth capacity in monolayer culture³. Here we present evidence that the v-myc oncogene alone is sufficient to induce growth transformation of glial and neuronal precursor cell types from chick neuroretina. We also report that induction of transformation by v-myc is accompanied by faithful preservation of some of the differentiated functions of the chick cells.

Dissociated neuroretina (NR) cells from 7-day chicken embryos, consist primarily of replicating neuroepithelial cells belonging to both the glial and neuronal lineages and post-mitotic neurons. As the proliferation state of a cell population may affect the efficiency of infection and subsequent spread of virus by re-infection, optimized culture conditions were established (see Fig. 1 legend), under which uninfected NR cells could be successfully cultivated for as many as 10–15 population doublings before cells completely ceased growing. To assess whether the v-myc-containing viruses MC29 and MH2 (which also carries the v-mil oncogene) can transform neural cells *in vitro*, NR cells were infected with MC29, MH2, PR-A (which carries the v-src oncogene) and the non-transforming Rous-associated virus 1 (RAV-1)⁴. Cultures were then passaged twice and assayed for the expression of transformation-associated traits. Third-passage, morphologically transformed NR cells, subcultured at low density, showed reduced doubling times and increased rates of DNA synthesis as compared with uninfected or RAV-1-infected cells of the same age *in vitro* (Fig. 1a). To determine the percentage of infected NR cells producing transforming virus at the same passage, RSV, MC29 and MH2-infected cells were treated with mitomycin C and tested for their ability to induce secondary transformed foci after replating on chicken embryo fibroblasts. The infectious centre tests revealed that at this stage the majority (>80%) of NR cells in culture produced RSV or MH2, whereas in the case of MC29 only 30% of cells were virus producers. The latter value may explain the kinetics in Fig. 1a. NR cells uniformly infected by MC29 and MH2 shed high numbers of cells into the medium after reaching confluence, maintained a sustained growth rate as shown by ³H-thymidine incorporation and cells could be efficiently grown *in vitro* for up to 50 population doublings. To determine whether virus-infected cells had acquired anchorage-independent growth properties, fifth-passage infected cells were seeded in soft-agar and colonies counted two weeks later. Table 1 shows that all viruses were capable of inducing anchorage independence, albeit with different efficiencies. Colonies produced by the MC29- and MH2-infected NR cells were larger and more

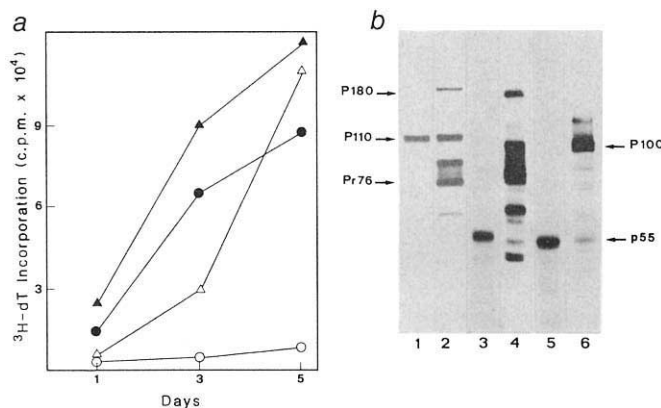


Fig. 1 a, Kinetics of DNA synthesis in NR cells infected with viruses. NR cells were infected with RAV-1 (○), PR-A (●), MC29 (△) and MH2 (▲), then passaged twice, seeded at 5×10^4 per well on collagenated clusters and finally labelled with ³H-dT as indicated below. b, Analysis of viral proteins in NR cells (5th passage) infected with MC29 (lanes 1, 2), MH2 (lanes 3, 4) and non-producer chick embryo fibroblasts transformed by MH2 (lanes 5, 6).

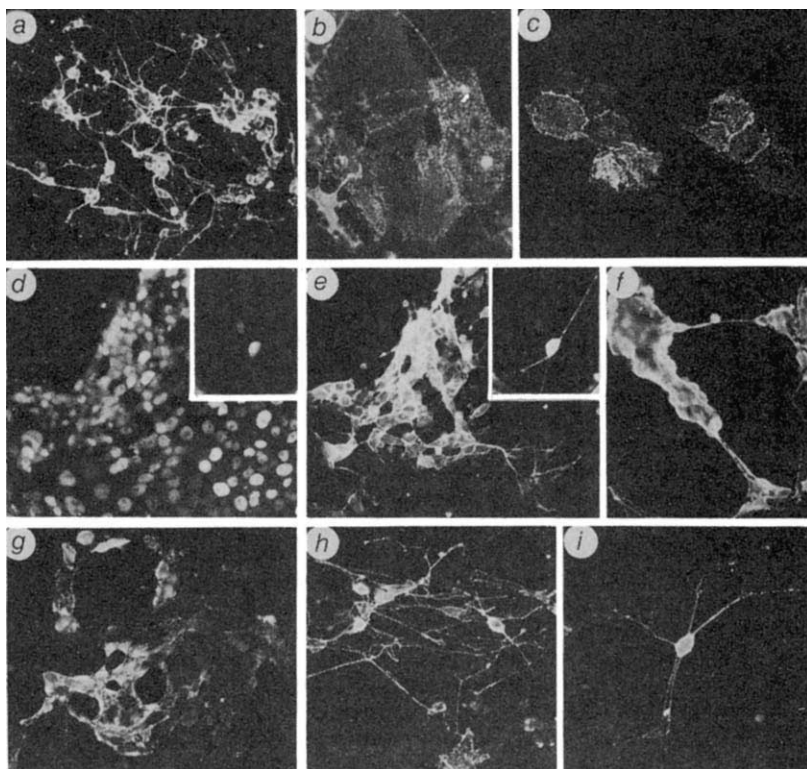
Methods. For transformation of NR cells and ³H-thymidine incorporation, neural retinae (NR) were dissected from 7-day-old chicken or quail (*Coturnix japonica*) embryos and cell suspensions prepared by using standard procedures. After washing, the cells were suspended in Dulbecco's modified Eagle's medium (DMEM, Flow) containing 0.2% fetal calf serum (FCS, Flow) and plated 5×10^6 cells per 60-mm collagen-coated (Vitrogen, Flow) tissue culture dish (Falcon) to promote rapid attachment to the substrate. The cells were thereafter cultured in growth medium (DMEM containing 10% FCS, 1% chicken serum, 10% triptose phosphate broth) on collagen-coated dishes and fed every other day. Primary cultures were infected at high multiplicity of infection (0.1–1.0) with the following viruses: RAV-1, PR-A, MC29(RAV-1) and MH2(RAV-1). The origin of the viral stocks is described in refs 23 and 24. Control and virus-infected cultures were maintained at 37 °C. Third-passage cultures were labelled for 2 h, at day 1, 3 and 5 after seeding, with ³H-thymidine (NEN, 77 Ci mmol⁻¹), using $2 \mu\text{Ci ml}^{-1}$ in growth medium supplemented with $2 \mu\text{M}$ cold thymidine. Following incubation, cultures were washed successively in cold phosphate-buffered saline (PBS), 5% trichloroacetic acid (TCA) and methanol; cells were then solubilized in 0.1 M NaOH and counted in Picofluor (Packard). For cell labelling and immunoprecipitation, fifth-passage, infected NR cells were washed twice with methionine-free MEM and then exposed to the same medium also containing $100\text{--}200 \mu\text{Ci ml}^{-1}$ of ³⁵S-methionine (NEN). Incubation was for 1 h at 37 °C. After labelling, cultures were washed twice with PBS and frozen *in situ* at -80°C until used for further analysis. Labelled cells for immunoprecipitation were collected and lysed in RIPA buffer³¹, also containing 0.2% Trasyolol, 0.2mM phenylmethylsulphonyl fluoride (PMSF) and 5 mM EDTA. After clarification at 13,000 r.p.m. for 30 min in a microfuge, aliquots of extracts, normalized for TCA-precipitable counts, were challenged with $3\text{--}5 \mu\text{l ml}^{-1}$ of either a serum raised in rabbits against a bacterially expressed viral myc protein (obtained from K. Moelling) (lanes 1, 3, 5) or an anti-gag serum (lanes 2, 4, 6) and the mixtures incubated on ice for 1 h. Immunoprecipitates were collected with Pansorbin (Calbiochem), washed and dissolved by boiling in SDS-sample buffer. Proteins were resolved by electrophoresis on 8% SDS-polyacrylamide gels³² followed by fluorography and autoradiography. Immunoprecipitates analysed in lanes 1 and 2 were run on a separate gel.

numerous than those produced by PR-A-infected cells. Qualitatively similar results were obtained in independent experiments carried out with quail embryo NR cells (data not shown). To analyse the relationship between induced growth transformation and levels of expression of oncogene products, MC29- and MH2-infected cells were labelled with ³⁵S-methionine and immunoprecipitated with the appropriate antibodies. Infected cells expressed levels of the putative transforming products⁵

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Fig. 2 Indirect immunofluorescent visualization of neural specific antigens in uninfected and virus-infected NR cells. *a-c*, Primary (*a, b*) or fifth passage (*c*) control retinal cells were stained with the monoclonal antibodies A2B5 (*a, b*) or Leu7 (*c*). *d, e*, Same field of 7th passage MC29-infected NR cells double-stained with both anti-*v-myc* serum (*d*) and monoclonal antibody A2B5 (*e*). The enlarged insets clearly show a cell with a neuronal phenotype stained by both antibodies. *f-i*, NR cells infected with (*f, g*) MC29 or (*h, i*) MH2 and stained with A2B5 (*f, h*) or Leu7 (*g, i*).

Methods. Uninfected or virus-transformed cells cultured on collagen-coated 35-mm dishes were labelled with A2B5 ascite fluid (1:50) (prepared from cells obtained from the American Type Culture Collection) or Leu7 (1:5) (from A. Mudge) and, after fixation for 10 min with 3.3% paraformaldehyde in PBS, stained with goat anti-mouse immunoglobulin antibodies conjugated to fluorescein (Miles, 1:30). For double-staining with A2B5 and anti-*v-myc* (1:50) the same slide was fixed as above, and permeabilized with 0.5% Triton X-100 (BioRad) in Tris-buffered PBS. Second antibodies were affinity-purified goat anti-rabbit antibodies conjugated to rhodamine (Zymed, 1:50) and goat anti-mouse immunoglobulin conjugated to fluorescein. After washing, cultures were mounted in Gelvatol and examined in a Leitz Dialux fluorescence microscope.



P110^{gag-myc}, p55^{myc} and P100^{gag-mil} which were comparable to those of infected fibroblasts (Fig. 1*b*). In addition we attempted to localize *v-myc* protein in infected cells by immunofluorescence of fixed cells. Antisera against bacterially expressed *v-myc* protein stained nuclei⁶ strongly in all MC29- (Fig. 2*d*) and MH2-infected (data not shown) NR cells.

We next attempted to characterize the lineages of NR-transformed cells. The neuroretina of the 6-7th-day avian embryo consists of cells with different capacities for self-renewal and differentiation^{7,8}. Progenitor cells that can be grown *in vitro* include presumptive glia cells of the Muller type⁹ and cells which, when seeded on the proper substrate, give rise to cells with a neuronal phenotype as defined by a round, refractile body with thin neurites¹⁰. NR cells infected by PR-A gave rise to a uniform population of moderately transformed cells capable of reverting to a flat, glial-like phenotype when shifted to the non-permissive temperature in *ts*-RSV-infected cells (data not shown and ref. 11). MC29- and MH2-infected cells were small and had prominent nucleoli, a hallmark of *v-myc*-transformed cells¹². Moreover, cultures infected with the latter viruses consisted of a carpet of epithelioid, flat cells underlying a population of process-bearing cells, with a contained body. MC29- and MH2-transformed NR cells continued to express the high-affinity, Na-dependent D-aspartate (a non-metabolized analogue of the neurotransmitter L-glutamate) and γ -aminobutyric acid (GABA) transport systems that have been described for NR cells in culture^{13,14}, whereas the same activities were largely inhibited by *v-src* (Table 1). Autoradiographic experiments showed that uptake of D-aspartate was a property of flat glia-like cells, but GABA uptake, as in primary cultures, was confined to a subset of neuron-like cells which had grown long processes (not shown).

Uninfected and virus-infected cells were also characterized for the expression of tissue-specific antigens. In Fig. 2*a*, a cluster of NR neurons from a primary culture, recognizable by their round cell body and extensive process formation, are shown on a background of flat non-neuronal cells. In *a* only the neurons were stained by monoclonal antibody A2B5, which recognizes a tetrasialoganglioside unique to neural cells¹⁵, and Leu7 which

Table 1 Agar colony formation and uptake of neurotransmitters by control and virus-infected NR cells

Virus	Agar colonies % efficiency	D-Asp uptake	GABA uptake
Uninfected	0	10.5	1.4†
RAV-1	0	8.6	n.d.
PR-A	10 ⁻⁴ *	0.9	0.5
MC29	2 × 10 ⁻³	25.8	9.2
MH2	10 ⁻²	16.2	4.1

* Large (>0.25 mm) colonies only.

† Low levels of GABA uptake in control cells are due to loss of neurons on passaging.

Uptake of D-Asp and GABA is expressed in nmol min⁻¹ per mg protein; n.d., not determined. Agar colony assays were performed on fifth-passage control and infected NR cells as described²⁴. Plates were scored for colonies 14 days after seeding. For the uptake determination and autoradiography, fifth-passage cells were rinsed twice with a HEPES-buffered medium (137 mM NaCl, 2.7 mM KCl, 1.14 mM KH₂PO₄, 6.45 mM Na₂HPO₄, 0.5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose) and then incubated for up to 30 min at 37 °C in ³H-D-aspartic acid (2 μ Ci ml⁻¹; specific activity 20 Ci mmol⁻¹, NEN) or ³H-GABA (2 μ Ci ml⁻¹; specific activity 33 μ Ci mmol⁻¹, NEN). After incubation the dishes were washed three times with warm medium and either fixed for autoradiography or solubilized in 0.1 M NaOH and counted in Picofluor (Packard). The dishes were processed for autoradiography as in ref. 30.

labels neuroectodermal cells¹⁶ (not shown). In primary cultures only a proportion of flat glia cells were stained by A2B5 (Fig. 2*b*) or Leu7 and this property was progressively lost *in vitro* so that <5% were positive at later passages (Fig. 2*c*). Double immunofluorescence studies (Fig. 2*d, e*) showed the simultaneous presence of P110^{gag-myc} protein and A2B5 antigen in a large proportion of flat and process-bearing MC29-transformed cells; as in uninfected primary cultures, a restricted fraction of neuron-like cells expressed the 70K subunit of neurofilaments (data not shown). Expression of these markers although maintained in process-bearing MC29- (Fig. 2*f, g*) and MH2-

transformed cells (Fig. 2*h, i*), was not detectable in PR-A-transformed cells (data not shown and ref. 17).

The present data provide strong evidence that in primary chick NR cells the *v-myc* oncogene does not require the action of a complementary oncogene such as *v-mil* to induce two major traits associated with loss of growth control in transformed cells. Although entirely consistent with the conclusion that the *v-mil* oncogene of MH2 appears non-essential for transformation of fibroblasts¹⁸ and macrophages¹⁹ *in vitro*, our results differ considerably from the experiments of Bechade *et al.*², where infection of chick NR cells with MC29 and deletion mutants of MH2 expressing only the *v-myc* oncogene had no apparent effect on these cells. Two points relevant to this issue may be made. The first suggests that part of the apparent discrepancy may have arisen from our improved culture conditions in which efficient virus spread allows a rapid and uniform viral infection. A second, complementary argument stems from our observation that in chick NR cells mitogenic stimulation by *v-myc*, in contrast to *v-src* or other oncogenes, becomes apparent only after expression of the transforming protein by the majority of the cells (Fig. 1*a* and data not shown). This behaviour is not observed if NR cells are infected with a retrovirus such as MH2 expressing both the *v-myc* and the *v-mil* oncogenes (this study and ref. 2). At a superficial level, this property suggests the existence of environmental cues that delay the expression of the transformed phenotype in MC29-infected NR cells.

That primary avian cells belonging to different lineages are, with no known exception, efficiently transformed *in vitro* by retroviruses carrying the *v-myc* oncogene alone²⁰⁻²⁴ contrasts with the observation that in non-established rodent fibroblasts transfected *v-myc* usually requires a cotransforming oncogene of the *ras* type^{25,26}. In one instance, a *v-myc*-containing recombinant retrovirus was able to confer anchorage-independence on non-established mouse fibroblasts and macrophages²⁷. A possible resolution for the incompletely transformed phenotype of *v-myc*-bearing mammalian cells may come by considering that (1) mammalian cells express comparatively lower levels of exogenous *v-myc*²⁷ and (2) normal adjacent cells appear to exert a 'suppressive' effect on the focal outgrowth of mammalian cells bearing *v-myc*²⁸. Accordingly, rare full transformation of rat embryo fibroblasts seems to require both elimination of adjacent cells and high levels of expression of the *v-myc* protein²⁸.

Interestingly, at the biochemical level, expression of *v-myc* did not appreciably reduce the high-affinity uptake of D-aspartate or GABA, even though the same activities were severely affected by *v-src*. Furthermore, the expression of markers of neurons and glia cells was not appreciably affected, except for changes that could be attributed to the high proliferation rate of transformed cells. Indeed, the specific activities of neurotransmitter uptake and the immunofluorescence studies suggest that, on passing, the *myc*-transformed cells retained their normal differentiated phenotype better than the uninfected cells. This is not surprising in view of previous work indicating that, in contrast to *v-src*, *v-myc* does not depend on blocking differentiation to promote transformation^{21-24,29}.

Regarding the origin of the cells acting as targets of MC29 and MH2 infection, at present we feel that the evidence is not sufficient to discriminate between infection of separate precursors or of a common progenitor to both glia cells and neurons. In any event, the feasibility of conferring proliferative potential on neuroectodermal cells without grossly interfering with their differentiated phenotype, suggests a novel experimental approach to generating clonal strains from short-lived, early neural precursor cells otherwise not easily amenable to adequate characterization.

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- Weinberg, R. A. *Science* **230**, 770-776 (1985).
- Bechade C. *et al.* *Nature* **316**, 559-562 (1985).
- Calothy G. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **44**, 983-990 (1980).
- Weiss, R. A., Teich, N. M., Varmus, H. E. & Coffin, J. M. (eds) *RNA Tumor Viruses* Vol. 2 (Cold Spring Harbor Laboratory, New York, 1982).
- Moelling, K. *Adv. Cancer Res.* **43**, 205-239 (1985).
- Bunte T. *et al.* *EMBO J.* **3**, 1919-1924 (1984).
- Kahn A. J. *Brain Res.* **63**, 285-290 (1973).
- Adler, R., Magistretti, P. J., Hyndman, A. G. & Shoemaker, W. J. *Dev. Neurosci.* **5**, 27-39 (1982).
- Crisanti-Combes, P., Privat, A., Pessac, B. & Calothy, G. *Cell Tiss. Res.* **185**, 159-173 (1977).
- Adler, R., Jerdan, J. & Hewitt, A. T. *Dev. Biol.* **112**, 100-114 (1985).
- Calothy, G. & Pessac, B. *Virology* **71**, 336-345 (1976).
- Palmieri, S., Kahn, P. & Graf, T. *EMBO J.* **2**, 2385-2389 (1983).
- Hyndman, A. G. & Adler, R. *Dev. Brain Res.* **3**, 303-314 (1982).
- Hyndman, A. G. & Adler, R. *Dev. Brain Res.* **3**, 167-180 (1982).
- Eisenbarth G. S., Walsh F. S. & Nirenberg M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913-4917 (1979).
- Schuller-Petrovic, S., Gebhart, W., Lassmann, H., Rumpold, H. & Kraft, D. *Nature* **306**, 179-181 (1985).
- Brackenbury, R., Greenberg, M. E. & Edelman, G. M. *J. Cell Biol.* **99**, 1944-1954 (1984).
- Zhou, R.-P., Kan, N., Papas, T. & Duesberg, P. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6389-6393 (1985).
- Graf, T. *et al.* *Cell* **45**, 357-364 (1986).
- Royer-Pokora, B. *et al.* *Cell* **13**, 751-760 (1978).
- Graf, T. & Beug, H. *Biochim. biophys. Acta* **516**, 269-299 (1978).
- Durban, E. M. & Boettiger, D. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3600-3604 (1981).
- Alemà, S., Tatò, F. & Boettiger, D. *Molec. cell. Biol.* **5**, 538-544 (1985).
- Falcone, G., Tatò, F. & Alemà, S. *Proc. natn. Acad. Sci. U.S.A.* **82**, 426-430 (1985).
- Land, H., Parada, L. & Weinberg, R. A. *Nature* **304**, 596-602 (1983).
- Moungneau, E., Lemieux, L., Rassoulezadegan, M. & Cuzin, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5758-5762 (1984).
- Vennstrom, B. *et al.* *EMBO J.* **3**, 3223-3229 (1984).
- Land, H., Chen, A. C., Morgenstern, J. P., Parada, L. F. & Weinberg, R. A. *Molec. cell. Biol.* **6**, 1917-1925 (1986).
- Alemà, S. & Tatò, F. *Adv. Cancer Res.* (in the press).
- Levi, G., Aloisi, F., Ciotti, M. T. & Gallo, V. *Brain Res.* **290**, 77-86 (1984).
- Brugge, J. S. & Erikson, R. L. *Nature* **269**, 346-348 (1977).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).

Retroviral transduction of T-cell antigen receptor β -chain and *myc* genes

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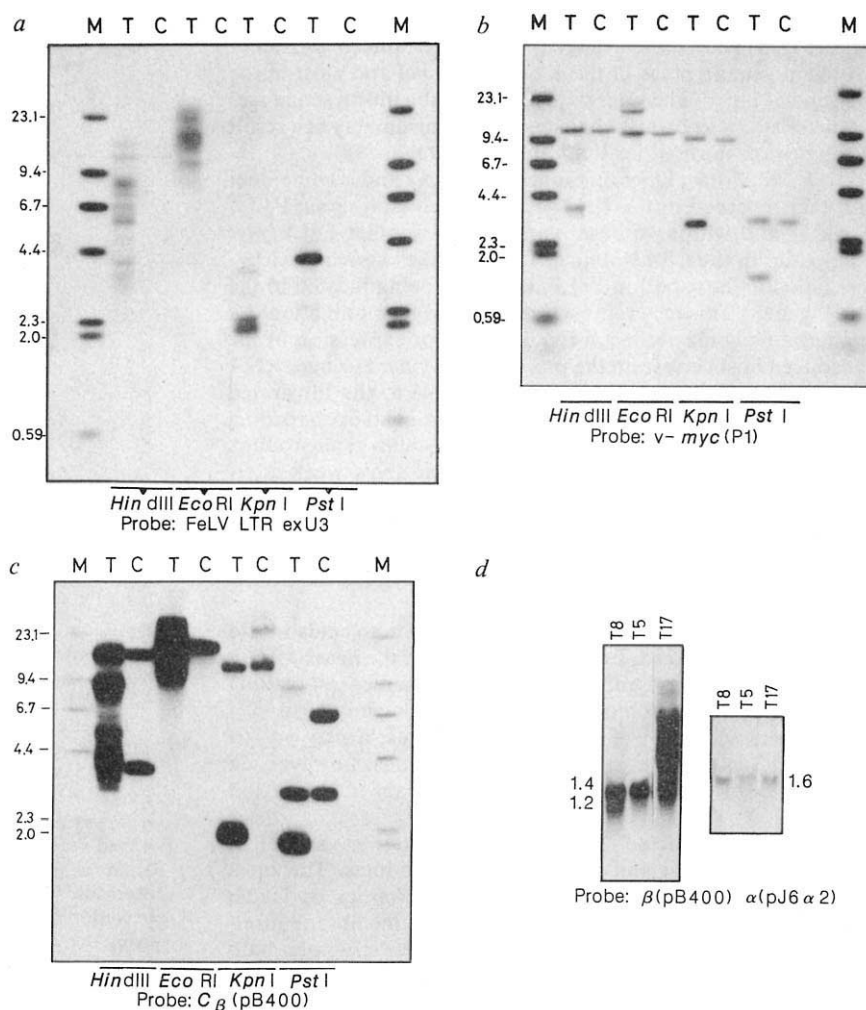
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Support for multistage models of oncogenesis has been provided by several highly leukaemogenic retrovirus isolates that have transduced more than one host cell gene¹⁻⁵. Where functional studies have been performed, these retroviral oncogenes show synergy for *in vitro* transformation and leukaemogenesis^{6,7}. In naturally occurring feline leukaemias associated with feline leukaemia virus (FeLV), retroviral transduction of *myc* is a frequent oncogenic mechanism⁸⁻¹⁰. But evidence suggesting that the FeLV *v-myc* genes might be insufficient for leukaemogenesis was provided by the latency (12 weeks) and clonality of FeLV/*v-myc*-induced tumours and the absence of demonstrable *in vitro* transformation by these viruses¹¹. In the search for secondary leukaemogenic events in FeLV/*v-myc* tumours, we have identified a case of FeLV transduction of a T-cell antigen receptor β -chain gene. The proviruses carrying this gene (which we have named *v- β*) were a separate population from those carrying *v-myc*. In its normal role, the T-cell receptor β -chain forms part of a multimeric complex involved in antigen recognition¹²⁻¹⁴ and T-cell activation^{15,16}. We suggest that *v- β* is a novel viral oncogene which assisted *v-myc* in the genesis of a naturally occurring case of thymic lymphosarcoma.

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Fig. 1 Evidence for FeLV proviruses carrying *myc* and T-cell antigen receptor β -chain genes in feline thymic lymphosarcoma T17. Restriction enzymes such as *KpnI* and *PstI* have conserved sites in FeLV long terminal repeats⁴² and generate common internal proviral fragments regardless of integration site. On digestion, multiple proviral copies yield a single strongly hybridizing restriction fragment. In contrast, *EcoRI* is generally a non-cutter in FeLV so that the fragment detected by Southern blot hybridization is different for each integrated copy but of greater length than the FeLV provirus. Hybridization was carried out with: *a*, an FeLV LTR probe from the U3/R region⁴³; *b*, a probe derived from exon 2 and 3 domains of FeLV CT4 v-*myc*^{8,20}; and *c*, a constant region fragment from a cDNA clone of human T-cell antigen receptor β -chain gene (pB400)¹⁹. In *a-c*, T denotes tumour DNA and C, control tissue (kidney) DNA; M, size markers (end-labelled λ HindIII fragments). *d*, Northern blot analysis of feline T-cell tumour RNAs for α - and β -gene transcripts. RNAs from thymic lymphosarcomas T5 and T8 are shown alongside T17 to exemplify the typical transcript patterns we found in a survey of ~30 tumours (ref. 11 and unpublished results).

Methods. For Southern blots in *a*, *b* and *c*, 20 μ g DNA for each lane was digested with the appropriate restriction enzyme. Samples were separated on 0.8% agarose gels and transferred to GeneScreen membranes (NEN). Hybridization was performed overnight at 42 °C in a solution containing 5 $\times$ SSC, 5 $\times$ Denhardt's solution, 0.1 M sodium phosphate, 0.1% SDS, 10% dextran sulphate and 100 μ g ml⁻¹ denatured salmon sperm DNA. The blots were washed at 60 °C for 1 h in 0.5% SDS and (*a*) 0.2 $\times$ SSC, (*b*) 2 $\times$ SSC and (*c*) 0.1 $\times$ SSC, before final rinsing, drying and exposure to Kodak XAR-5 film at -70 °C. For the Northern blot analysis in *d*, 20 μ g samples of total cellular RNA were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde, transferred onto GeneScreen membranes and hybridized with α - and β -chain gene probes essentially as for Southern blots. Filters were washed in 2 $\times$ SSC, 0.5% SDS at 60 °C (3 $\times$ 25 min). The transcript sizes were estimated relative to radiolabelled HindIII-digested λ DNA markers (not shown). Membranes were exposed to Kodak XAR film at -70 °C for 24 h (T_β) or 48 h (T_α).



Tumour T17 was a field case of thymic lymphosarcoma in a young adult male cat. The case was unusual in that the cat had no demonstrable FeLV viraemia or viral antigen in blood plasma, although high-titre FeLV-neutralizing antibodies were present. The tumour cells harboured multiple FeLV proviruses which were highly expressed as intracellular RNA and viral protein (Fig. 1 and unpublished results). The tumour cells were readily established in culture with no requirement for exogenous interleukin-2 (IL-2), but released only very low titres of infectious FeLV (less than 10 focus-forming units (f.f.u.) per ml). This pattern of *in vitro* growth without IL-2 was characteristic of other T-cell tumours induced by FeLV/v-*myc* viruses¹¹, and we found that T17 tumour DNA also harboured FeLV recombinant proviruses carrying the *myc* gene (Fig. 1b).

Our evidence for derangement of a T-cell antigen receptor (*Ti*) gene in tumour T17 came from a larger study in which we assessed the phenotype and maturity of a series of feline T-cell tumours by hybridization analysis of the rearranging genes (α -chain and β -chain) of the T-cell receptor complex. The α - and β -chain genes are sequentially rearranged and expressed during thymocyte ontogeny (β then α)¹⁷⁻¹⁹. These studies were facilitated by the finding that the feline *Ti* genes can be detected readily using probes derived from the human *Ti* genes and that the feline genes have similar structural organization and transcript sizes to the human genes. Most of the feline thymic tumours examined, notably those involving altered *myc* genes

(c-*myc* or v-*myc*) expressed both α - and β -gene transcripts of the expected size and demonstrated clonal patterns of β -gene rearrangement. Tumour T17, however, showed around tenfold amplification of β -chain DNA sequences; further restriction mapping provided preliminary evidence that FeLV proviral capture was the basis for this amplification (Fig. 1c). Also, the β -gene transcripts were much larger and more abundant than normal (Fig. 1d), again consistent with their expression as part of an FeLV provirus. In contrast to β , the expression of the α -gene in T17 appeared to be normal (Fig. 1d).

For more detailed analysis, we generated a bacteriophage library from T17 DNA and screened with probes including C_β (human β -chain constant region)¹⁹, v-*myc*^{8,20} and an exogenous FeLV-specific *env* gene probe²¹. By this approach we have cloned FeLV proviruses carrying the β -chain sequences, proviruses carrying v-*myc* and other proviruses that appear to be typical helper-type FeLV. None of the clones hybridized to both *myc* and C_β , confirming the prediction from Southern blot analysis that the two host genes were on separate proviral elements (Fig. 1).

The clones T17T-22 and T17T-31 contained indistinguishable defective FeLV proviral structures incorporating the C_β -hybridizing sequence at the 3' end in place of *env* sequences (Fig. 2). Their internal structures were as expected from Southern blot analysis of tumour DNA (Fig. 1c). A 1.9-kilobase (kb) *PstI* fragment of T17T-22 was subcloned into plasmid

pUC18 for DNA sequence analysis. The sequence of this fragment (Fig. 3) shows a rearranged, joined and spliced version of a β -chain gene in place of the 3' end of the *pol* and most of the *env* gene of FeLV. The *v-tcr* sequence lacks the intron sequences characteristic of known β -chain gene loci, presumably as a result of replication through an RNA intermediate.

The 5' FeLV-host junction point is within *pol* and is coincident with that recorded in the FeLV/*myc* recombinant virus, FTT²² (Fig. 3b) and within 16 base pairs (bp) of another FeLV/*myc* 5' junction, in the CT4 isolate²⁰. This identifies a second virus-host junction 'hot-spot' in FeLV, the other being located in the p30^{gag} gene²³. This may represent a favoured recombination site or alternatively may reflect a requirement for expression of the transduced host gene from the presumptive *env* messenger RNA splice acceptor site (at position -15 relative to the illustrated sequence). The acquired host sequence includes an open reading frame of 963 bp that encodes a full-length β -chain gene product. Sequences 3' to the *v-tcr* open reading frame show weak interspersed homology to murine C_{β} 3' untranslated sequences^{24,25}. A better match can be obtained to $C_{\beta 2}$ than to $C_{\beta 1}$ but because there is such divergence between all three sequences, direct analysis of the feline C_{β} genes will be required to establish the precise genetic origin of *v-tcr*.

A full-length β -chain gene product of 331 amino acids would be encoded by a spliced, subgenomic mRNA if the nearby FeLV splice acceptor site is used. Otherwise, the presumed *pol* reading frame (identified by homology to murine leukaemia virus^{20,26}) is coincident with that of the *v-tcr* gene. Thus, a *gag-pol-tcr* fusion product may be generated. This depends, however, on the integrity of the *pol* reading frame upstream of the region sequenced.

Within the predicted FeLV *v-tcr* product, we see close homology to human and murine β -gene products. The open reading frame encodes a hydrophobic N terminus or leader sequence, which may be expected to mediate membrane insertion, followed by a sequence which shows clear structural hallmarks of a V_{β} gene product. This is shown in Fig. 4 as an alignment with a murine V_{β} gene product. A strikingly close alignment is possible with a murine V_{β} sequence obtained from BALB/c thymocytes (TB21)²⁷. The *v-tcr* V domain falls into the class I V_{β} genes defined by Schiffer and colleagues²⁸, and has all the expected conserved sequence hallmarks presumed to be important for V_{β} secondary structure and the generation of the antigen binding site.

Much of the diversity of β -chain sequences that should account for immunological specificity appears to be generated by V-D-J joining²⁷⁻³⁰. Sequences that appear to be derived from D and J elements are also seen in FeLV *v-tcr*. The derived amino-acid sequence from the presumptive J_{β} segment also shows good alignment with human and mouse sequences. The best match is to the murine $J_{\beta-1(86T1)}$ sequence^{24,31} (Fig. 4). The D_{β} coding sequences are intrinsically more variable, presumably because of the small size of the core elements and the possibility of nucleotide additions during gene rearrangement²⁷⁻³⁰. However, because the *v-tcr* V_{β} and J_{β} homologous regions are interrupted, we expect that the interposed sequence was derived from a D_{β} element.

The C-terminal portion of the predicted *v-tcr* product shows even closer homology to human and murine C_{β} products^{24,25,32,33} (Fig. 4). The differences between the *v-tcr* and human C_{β} sequences are slightly fewer than those between human and mouse C_{β} and no more than would be expected between the germline genes of these different animal species. Again, the important sequence hallmarks such as the cysteine residues are conserved, as is the putative C_{β} transmembrane domain at the C terminus.

There is a difference in the transmembrane domain which may be important when considering the possible transforming potential of the *v-tcr* gene. This is a non-conservative change in *v-tcr* relative to the human and mouse coding sequences

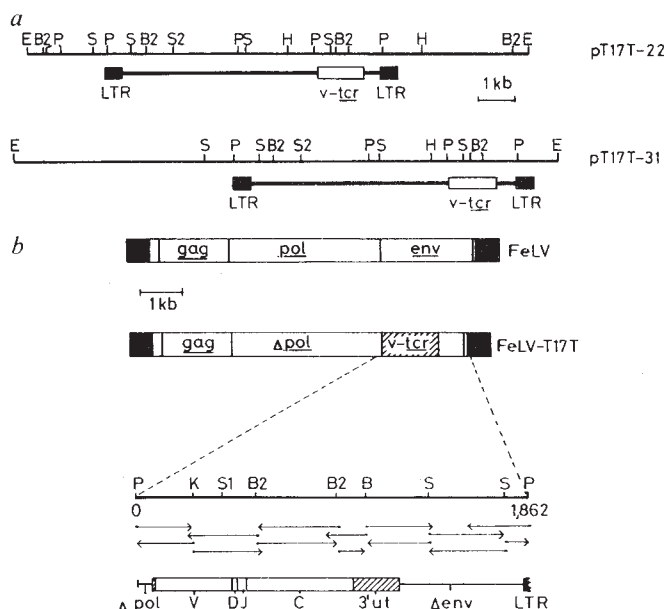


Fig. 2 a, Restriction maps of selected molecular clones. Line below map, position of FeLV genome with (black boxes) LTR regions and (open box) *v-tcr* gene. Restriction enzyme sites shown: B2, *Bgl*III; E, *Eco*RI; H, *Hind*III; S1, *Sst*I; S2, *Sst*II and P, *Pst*I. b, Genetic location of the β -chain gene sequences within FeLV T17T. A typical replication-competent FeLV (dimensions based on FeLV-A/Glasgow-1²¹) is shown above for comparison. The 1.9-kb *Pst*I fragment is also shown in larger scale below, with (arrows) sequencing strategy and the coding potential of the host-driven sequence indicated by boxes. Restriction enzyme abbreviations as in a, except B, *Bam*HI; K, *Kpn*I.

Methods. To clone the novel proviral elements from T17 DNA, a bacteriophage library was constructed from DNA of tumour T17 using the *Eco*RI cloning vector λ EMBL4 (Northumbria Biologicals). Tumour DNA was digested to completion with *Eco*RI, and size-selected (10–20 kb) on low-melting-point agarose gels before ligation to phage arms. Around 1×10^6 recombinants were obtained from 2 μ g of size-selected DNA. Several probes were used to screen the library, including the C_{β} probe, an FeLV *v-myc* probe, an FeLV LTR (U3) probe, and an exogenous FeLV-specific *env* gene probe²¹. Cross-hybridization analysis showed that most of the clones selected with *myc* and C_{β} probes also hybridized to FeLV LTR but not to *env* gene probes. The *Eco*RI bacteriophage vector inserts were subclones into pUC18 and mapped by double digest and partial digest analysis. The location and orientation of FeLV and β -chain gene sequences were deduced from hybridization analysis of the cloned DNAs and the presence of characteristic restriction enzyme cleavage sites.

(Met $\rightarrow$ Lys 325). Although this substitution does not alter the predicted α -helical structure, the charge alteration results in a significantly altered hydrophilicity profile. We draw comparison with the *neu* oncogene, a homologue of the epidermal growth factor receptor, where activation for *in vitro* transformation has been reported to result from a point mutation (Val $\rightarrow$ Glu) in the transmembrane coding region³⁴. In the human T-cell receptor (CD3(T3)-Ti) complex, there is normally a balance of charges within the transmembrane domain (three positive, three negative³⁵⁻³⁹): adding an additional positive charge (Lys) could conceivably alter intermolecular interactions in the complex.

The occurrence of a transduced host gene within a retrovirus in a naturally occurring tumour does not in itself constitute proof of a direct oncogenic function, although precedent strongly suggests that this is the case. When considering possible functions of the FeLV *v-tcr* gene product in oncogenesis, we must consider the properties of the T-cell antigen receptor complex. In this multimeric structure, two predominantly extracellular chains (α and β) are required for antigen recognition¹²⁻¹⁴ and several other transmembrane proteins (γ ,

b

5' Junction

FeLV	AAGCTCGAAACCCCGGTGCTGCCTACCCCTTCAAACGAC	
CT8		
CT4	AAGCTCGAACCCC	- FeLV
	gaaggagataCCGGAGTGACCG	- v- <u>myc</u>
T17T	AAGCTCGAAACCCCGGTGTGCCCCACCCCT	- FeLV
	GAGCTGGAT	- v- <u>tcr</u>
FTT	AAACTTGAAACCCCGGTGTGCTCATCCCT	- FeLV
	cggacgctGGATTTCCT	- v- <u>myc</u>

3' Junction

C β 2	GAACAGACTAAATCAATAAAAAATCATGGAGTTAACCTGGT	
v- <u>tcr</u>	GAATTTGACTGAATCAATAAAAAATGGCCATGCACACAGAC	
FeLV	AGCCCGATTGACACAACTACAAATGGCCATGCACACAGAC	
(G1)		

Methods. For sequencing, a 1.9-kb *Pst*I fragment of FeLV T17-22 (Fig. 3) was subcloned into plasmid pUC18, before further subcloning into M13 mp18 and mp19⁴⁴ and sequencing by the dideoxy method⁴⁵. Sequence was determined unambiguously on both strands using the cloning sites indicated. An open reading frame for a potential β -chain T-cell antigen receptor gene product of 331 amino acids spans nucleotides 93–1056.

How might the FeLV *v-ter* gene product disrupt the normal functioning of the receptor complex? We favour a model in which the FeLV *v-ter* product is expressed as a component of the receptor complex which it activates in the absence of antigen binding. However, it is possible that the FeLV *v-ter* product may be altered in structural location, conformation and/or transport to the cell surface. All of these properties may be changed if the gene product is expressed as a *gag-pol-ter* fusion protein. From studies on the requirements for *in vitro* proliferation of T lymphocytes it seems that triggering through the antigen receptor complex can induce an IL-2-dependent autocrine pathway⁴⁰. Also, *v-myc* genes release murine cytotoxic T-cell lines from dependence on IL-2⁴¹. These findings suggest

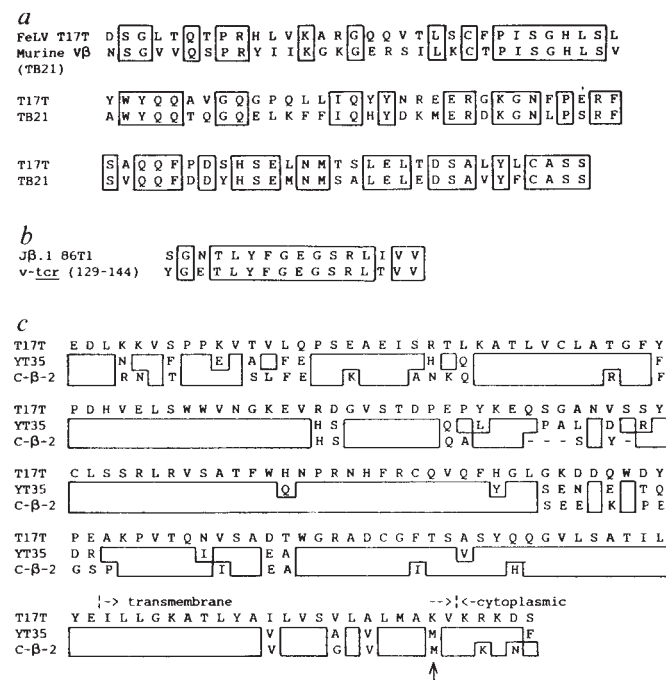


Fig. 4 Alignment of FeLV T17T-22 v-*tcr* predicted amino-acid sequence with β -chain gene products. **a**, Murine V_β gene product²⁴ is aligned with the homologous region of v-*tcr* (residues 29-122): 57% of the amino acids can be matched allowing only single amino-acid insertions and deletions. If conservative amino-acid substitutions are scored as matches, identity is 69%. **b**, Comparison of v-*tcr* (129-144) with a murine J_β coding sequence; **c**, three-way comparison of representative human³³ and murine³² C_β sequences with v-*tcr*. Open boxes, matches to v-*tcr*. Also indicated are the putative transmembrane and cytoplasmic domains of these gene products and a potentially important difference in the transmembrane region (arrow under sequence).

a possible cooperative role for v-*tcr* and v-*myc* in leukaemogenesis. We propose that the FeLV v-*tcr* gene in tumour T17 stimulates the antigen-receptor complex, and the FeLV v-*myc* gene augments the response or releases it from feedback regulation. Further studies will assess the possible oncogenic functions of v-*tcr*, *in vivo* and *in vitro*, alone and in combination with v-*myc*.

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- Vennstrom, B. & Bishop, J. M. *Cell* **28**, 135-143 (1982).
- Jansen, H. W., Ruckert, B., Lutz, R. & Bister, K. *EMBO J.* **2**, 1969-1975 (1983).
- Kan, N. C., Flordellis, C. S., Mark, G. E., Duesberg, P. H. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3000-3004 (1984).
- Nunn, M. F., Seeburg, P. H., Moscovici, C. & Duesberg, P. H. *Nature* **306**, 395-397 (1983).
- Leprince, D. *et al. Nature* **306**, 391-394 (1983).
- Frykberg, L. *et al. Cell* **32**, 227-238 (1983).
- Graf, T. *et al. Cell* **45**, 357-364 (1986).
- Neil, J. C. *et al. Nature* **308**, 814-820 (1984).
- Levy, L. S., Gardner, M. B. & Casey, J. W. *Nature* **308**, 853-856 (1984).
- Mullins, J. I., Brody, D. S., Binari, R. C. Jr & Cotter, S. M. *Nature* **308**, 856-858 (1984).
- Onions, D., Lees, G., Forrest, D. & Neil, J. C. *Int. J. Cancer* (in the press).
- Meuer, S. C. *et al. Nature* **303**, 808-810 (1983).
- Dembic, Z. *et al. Nature* **320**, 232-238 (1986).
- Yague, J. *et al. Cell* **42**, 81-87 (1985).
- Samuelson, L. E., Harford, J. B. & Klausner, R. D. *Cell* **43**, 223-231 (1985).
- Imboden, J. B., Weiss, A. & Stobo, J. D. *Immun. Today* **6**, 328-331 (1985).
- Royer, H. D. *Cell* **39**, 261-266 (1984).
- Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. *Nature* **314**, 103-106 (1985).
- Collins, M. K. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 4503-4507 (1985).
- Stewart, M. *et al. Virology* **154**, 121-134 (1986).
- Stewart, M. *et al. J. Virol.* **58**, 825-834 (1986).
- Doggett, D. L. *et al. J. Virol.* (submitted).

- Besmer, P. *et al. Nature* **308**, 415-421 (1986).
- Malissen, M. *et al. Cell* **37**, 1101-1110 (1984).
- Gascoigne, N. R. J., Chien, Y., Becker, D. M., Kavaler, J. & Davis, M. M. *Nature* **310**, 387-391 (1984).
- Herr, W. J. *Virology* **49**, 471-478 (1984).
- Barth, R. K. *et al. Nature* **316**, 517-523 (1985).
- Schiffer, M., Wu, T. T. & Kabat, E. A. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4461-4463 (1986).
- Belhke, M. A. *et al. Science* **229**, 566-570 (1985).
- Leiden, J. M. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4456-4460 (1986).
- Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
- Goverman, J. *et al. Cell* **40**, 859-867 (1985).
- Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
- Bargmann, C. I., Hung, M.-C. & Weinberg, R. A. *Cell* **45**, 649-657 (1986).
- Krissansen, G. W., Owen, M. J., Verbi, W. & Crumpton, M. J. *EMBO J.* **5**, 1799-1808 (1986).
- Gold, D. *et al. Nature* **321**, 431-434 (1986).
- Tunnacliffe, A., Sims, J. E. & Rabbitts, T. H. *EMBO J.* **5**, 1245-1252 (1986).
- Chien, Y.-H. *et al. Nature* **312**, 31-35 (1984).
- Saito, H. *et al. Nature* **312**, 36-40 (1986).
- Meuer, S. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1509-1513 (1984).
- Rapp, U. R., Cleveland, J. L., Brightman, K., Scott, A. & Ihle, J. N. *Nature* **317**, 434-438 (1985).
- Mullins, J. I., Casey, J. W., Nicolson, M. O., Burck, K. B. & Davidson, N. in *Proceedings of the Third International FeLV Symposium* (eds Hardy, W. D. Jr, Essex, M. & McClelland, A. J.) 373-380 (Elsevier, New York, 1980).
- Casey, J. W. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 7778-7782 (1981).
- Norlander, J., Kempe, T. & Messing, J. *Gene* **26**, 101-106 (1983).
- Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. J. *molec. Biol.* **143**, 161-178 (1980).

Homozygotes for Huntington's disease

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Careful comparison of symptomatic individuals with normal controls has revealed the primary biochemical abnormality in many human genetic diseases, particularly recessive disorders¹. This strategy has proved less successful for most human disorders which are not recessive, and where a single copy of the aberrant gene has clinically significant effects even though the normal gene product is present. An alternative approach that eliminates the impediment of a normal protein in affected individuals is to study homozygotes for the mutant allele². For virtually all dominant human disorders in which homozygotes have been described, symptoms have been significantly more severe in the homozygote than in the heterozygote³. Thus, these disorders do not conform

to the classical definition of dominance which states that homozygotes and heterozygotes for a defect are phenotypically indistinguishable³⁻⁵. Instead, they display incomplete dominance, indicating that the normal allele may play a role in ameliorating the disease process. The *D4S10* locus, defined by the probe G8 and linked to the gene for Huntington's disease (HD), has permitted us to identify individuals with a high probability of being homozygous for this autosomal dominant neurodegenerative disorder⁶⁻⁹. These homozygotes do not differ in clinical expression or course from typical HD heterozygotes. HD appears to be the first human disease of genetically documented homozygosity that displays complete phenotypic dominance.

The HD gene was localized, in part, by study of the largest known HD family in the world, living along the shores of Lake Maracaibo, Venezuela¹⁰⁻¹³. The pedigree currently numbers almost 7,000 individuals: over 100 living symptomatic individuals and almost 900 family members born at 50% risk for carrying the gene defect but who are as yet asymptomatic. Among present generations of the pedigree, there are six unions between two symptomatic individuals, but there is nothing clinically unusual in their 31 offspring. We have concentrated our efforts on one large nuclear family, the 'H' family, in which both parents are living and many relatives could be assigned unequivocal genotypes.

To minimize the potential for bias in evaluating them, potentially homozygous offspring in the H family have been compared with 93 affected individuals, including 6 with juvenile onset of symptoms, and 307 'at-risk' relatives living in the same environment. Neurological, intellectual and behavioural function, as described in Fig. 1 have been assessed annually since 1981¹⁴.

Six years ago 1 of 14 children was already mildly symptomatic and in 1985 a second member of the sibship was diagnosed with HD after displaying 'soft signs' for the previous five years. The age of onset and progression of symptoms in these two is typical compared to those in other HD patients in Venezuela and the United States^{14,15}. In 1986, 7 siblings had soft signs and 5 remained neurologically normal.

The potential effects of homozygosity for the HD gene on intellectual function were also evaluated. A subset of the Venezuelan pedigree has been evaluated annually with a battery of neuropsychological tests assessing cognitive ability, memory and visual-motor dexterity¹⁶⁻²⁰. The offspring of the H family have not shown any unusually precipitous decline on neuropsychological tests. The H family is also typical with respect to the behavioural disturbances which are often noted in HD, as assessed through structured and unstructured interviews²¹⁻²³.

The *D4S10* locus was originally defined by the G8 probe, a 17-kilobase (kb) fragment of genomic DNA that detects two polymorphic *Hind*III sites when hybridized to human genomic DNA^{6,8}. A number of additional restriction fragment length polymorphisms (RFLPs) have been found using subclones of G8 or of R7, a second genomic clone overlapping G8^{7,9}. The probes used in this study, along with the polymorphic sites and haplotypes they define, are shown in Fig. 2. Results from typing the H family for these sites are shown in Fig. 1a and b.

The HD gene usually segregates with the C1 haplotype at the *D4S10* locus in the Venezuelan pedigree. Reconstruction of the *D4S10* haplotypes of all 4 grandparents of the H sibship confirms that each H parent inherited the HD gene on a chromosome with the C1 haplotype. In addition, the mother received the A3 haplotype and the father the A5 haplotype from their respective unaffected parents. In the absence of recombination, their offspring homozygous for the HD gene would also be homozygous for the C1 haplotype at the *D4S10* locus.

All four possible *D4S10* genotypes are represented among the 14 children of the H family. Four received two copies of the C1 haplotype, suggesting they are homozygous at the HD locus. Six children received one copy of the C1 haplotype, making them probable heterozygotes for the HD gene, and the remaining 4 children did not inherit the C1 haplotype. This

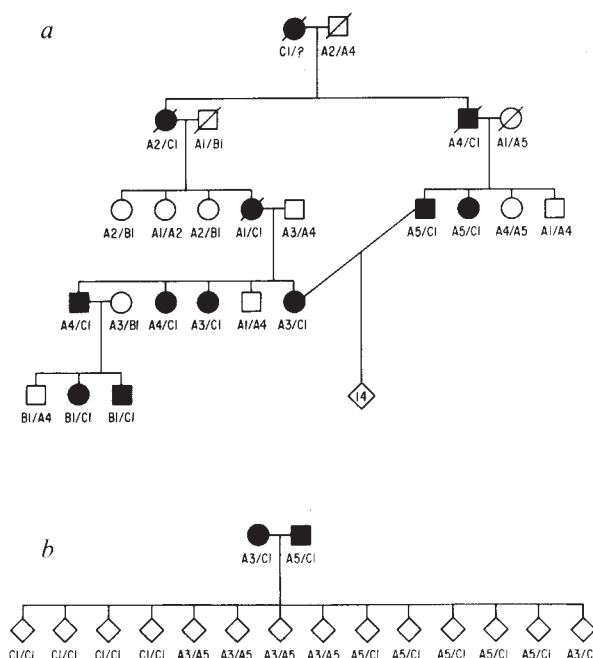


Fig. 1 Phenotypic assessment and segregation of *D4S10* and HD in the H family. *a*, Individuals from five generations of the H pedigree were typed for RFLPs at the *D4S10* locus. Family members diagnosed with HD are shown by darkened symbols and deceased individuals are denoted by a slash through the symbol. (Symbols are $\square$, female; $\square$, male; $\diamond$, one or more individuals, sexes not shown.) Haplotypes for *D4S10* were assigned as in Fig. 2. Genotypes were inferred from the phenotypic data after deducing the coupling phase of the various alleles for the *D4S10* RFLPs. In many cases, additional related individuals not shown here (for example, spouse and children) helped to confirm the assigned coupling phase. *b*, Inferred genotypes for the progeny of the HD by HD mating from *a*. The exact birth order of the children and the sex and HD status of each are not given to maintain confidentiality. Each C1/C1 individual has an approximately 92% probability of being homozygous for the genetic defect resulting in Huntington's disease.

Methods. The parents of the H family are second cousins once removed. The mother states that she is approximately 58 and her partner 63 years of age; no birth records exist. Each has been symptomatic for about 15 years. The mother began her pregnancies in her mid-teens with two miscarriages. In close succession, the couple had 15 children, including two sets of twins, one set of fraternal male twins and the other female. A female twin died at age 5 of gastroenteritis, leaving 14 living children available for study, ranging in age from 16 to 42 years. Blood samples from both parents, all 14 children and several other close relatives have been tested with more than 20 red-blood-cell and plasma markers and more than 50 polymorphic DNA markers to eliminate the possibility of non-paternity. Diagnosis of HD was based solely on the presence of characteristic extrapyramidal motor abnormalities. Behavioural disturbances were noted but were not themselves considered sufficient. The same neurologist (A.Y.) examined all members of the H family each year, joined in some years by between one and three other neurologists. Using the same rating scale, the neurologists demonstrated high inter-rater reliability in scoring³². The neurological examination assesses ocular motor ability, motor impersistence, fine motor coordination, tendon reflexes, and abnormal movements and postures such as chorea, dystonia and parkinsonian symptoms¹⁴. Functional capacity in patients was also rated³³. About one-third of the at-risk individuals examined displayed soft signs, minor neurological irregularities in one or more of the areas assessed. A diagnosis of HD was only made when unequivocal signs were present. Diagnostic feedback is not given unless requested, which almost never happens. Research participants correctly understand that the study is long-range and that its goals are to learn the cause of the illness and to devise therapeutic interventions.

distribution of progeny genotypes is consistent with mendelian segregation, assuming equal viability. It differs significantly ($P < 0.01$) from the distribution expected if HD homozygotes die prenatally.

We cannot be absolutely certain that the 4 children homozygous for the C1 haplotype are homozygous for the HD gene because of the possibility of recombination in the gametes of either or both parents. The genetic distance between the two loci is ~ 4 centimorgans (4% recombination, 1 lod confidence interval = 1–8%). Consequently, each child homozygous for the C1 haplotype has an $\sim 92\%$ chance of being homozygous for HD. The probability that none of the 4 is homozygous for the defect is $(0.08)^4$ or 4×10^{-5} suggesting that homozygosity at the HD locus does exist in this family and is not lethal *in utero*.

We have chosen to omit exact correlations between the genotypes in Fig. 1b and the personal and neurological data on individual members of the sibship, but this does not affect the conclusions to be drawn from this study. First and foremost, we wish to protect the confidentiality of all family members, particularly those found to be homozygous. Of the 14 offspring, 10 are destined to become ill but 4 should remain normal. The fact that children of HD homozygotes are inevitably HD-gene carriers makes privacy all the more essential. A second reason for avoiding identification of the individual genotypes in this family is our desire to continue the prospective study with investigators in the field remaining blind to the genetic status of each subject.

Among the 4 potential homozygotes for the HD gene there is at least one representative of each of the three diagnostic categories: definitive HD, displaying soft signs, and neurologically normal. There appear to be no phenotypic differences, either in age of onset, symptoms, or progression of illness, between an affected probable HD homozygote and the typical HD heterozygotes from the Venezuelan kindred or any US sample of patients. Similarly, a potential HD homozygote who displays soft signs or is neurologically normal cannot be distinguished clinically from other at-risk individuals who are potential HD heterozygotes. In terms of the phenotypes currently being monitored by neurological and psychological examinations, HD displays complete dominance. This permits two conclusions concerning the motoric, cognitive and psychiatric symptoms of this disorder: first, they are not influenced by dosage of the defective gene; and second, they are not mitigated by the normal allele.

Matings between two heterozygotes for a dominant disease are so rare that the opportunity to study large numbers of progeny from such unions is exceptional^{13,24–27}. In virtually every instance in which children are conceived between two heterozygotes, presumed homozygotes are quantitatively and sometimes qualitatively more severely affected than typical heterozygotes^{3,24–27}. The only possible exception to this rule described previously involved a family with Best's disease, or hereditary macular degeneration²⁸, where unfortunately, homozygosity could not be confirmed genetically.

In view of the obvious dose effects displayed by putative homozygotes in other dominant diseases, our finding that homozygosity for the defective allele in HD does not result in a more severely affected phenotype than heterozygosity is surprising. The importance of this observation lies in the clues it may provide to the nature of the disease process and its primary lesion. Complete dominance is known to exist in *Drosophila* and is thought to be associated with mutations causing a 'gain of function'^{29–31}. Clearly, HD cannot involve elimination of an essential enzyme activity as homozygosity is not lethal. The defect may compromise an enzyme activity that must exceed a strict threshold. Alternatively, the mutation might confer a novel property on a structural or regulatory protein, as the simple elimination of normal function would be expected to show a dosage effect or even lethality. If the HD defect causes a quantitative alteration, then overproduction of a normal protein, or

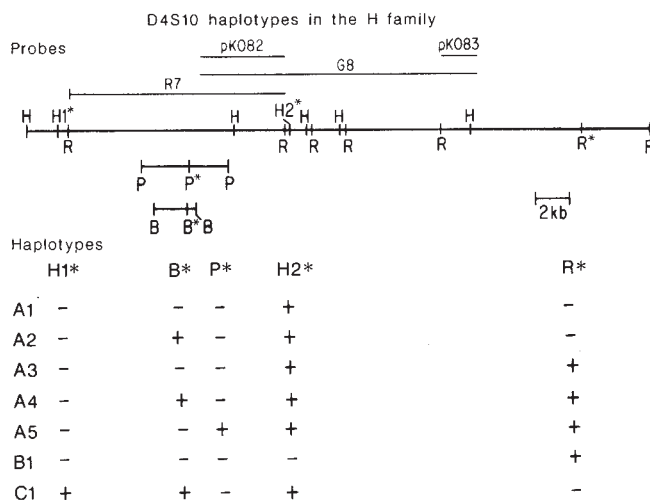


Fig. 2 *D4S10* haplotypes in the H family. A map of the polymorphic sites (denoted by *) and the probes used to detect them is shown (above) with definition of *D4S10* haplotypes (below); G8 is an insert of single-copy human DNA, from the terminal region of the chromosome 4 short arm, cloned in the phage vector Charon 4A^{6,7}. R7 is an overlapping genomic fragment also cloned in Charon 4A, and pK082 and pK083 are subclones of the indicated *EcoRI* fragments in pBR328. Two polymorphic *HindIII* (H) sites and one polymorphic *PstI* (P) site were detected using pK082 as a probe. Single polymorphic sites with *BglI* (B) and *EcoRI* (R) were monitored by probing with R7 and pK083 respectively.

Methods. For RFLP typing, genomic DNA from each individual was digested with the indicated enzyme (New England Biolabs), fractionated by agarose gel electrophoresis, transferred to a nylon membrane (Genatran, Plasco Inc.), and hybridized with ³²P-labelled probe DNA as described⁶, except that probes were prepared by oligonucleotide priming³⁴. Genomic DNA was obtained from permanent lymphoblastoid cell lines established for each member of the family as previously described^{35,36}. The presence or absence of each site on a given chromosome was used to define haplotypes at the *D4S10* locus. When all sites were considered, 7 haplotypes were evident in this portion of the Venezuelan HD pedigree and the disease segregated with the C1 haplotype.

the inappropriate production of a protein usually limited to a different tissue or to a different time of development, may be more likely than underproduction of a protein.

'Gene therapy' as a possible treatment for inherited disorders has engendered considerable interest recently. The normal allele at the HD locus does not apparently alter disease expression, so particularly imaginative approaches to gene therapy may be required for this condition: merely introducing a second normal allele is unlikely to have beneficial effects. Gene therapy aimed at blocking expression of the mutant allele (for example, by antisense mRNA) or at the introduction of unrelated gene(s) capable of correcting the biochemical defect may be a more rewarding approach.

The identification of specific genes or novel drugs capable of preventing the deleterious effects of HD is unlikely until the disease gene has been cloned and characterized. An intensive effort is underway to isolate the HD gene based on its map position despite limited knowledge of the nature of the defect. Cultured cells from HD homozygotes are very valuable in certain cloning strategies because their DNA contains no normal allele. Tissue samples from these individuals may ultimately enable identification of the gene product responsible for HD. Delineation of the molecular defect has obvious clinical importance and may also provide new insights into the biology of the nervous system. Of greater fundamental interest, perhaps, is that elucidating HD at the molecular level will reveal for the first time a genetic mechanism in humans capable of producing a completely dominant phenotype.

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- McKusick, V. A. *Mendelian Inheritance in Man* 6th edn (Johns Hopkins University Press, Baltimore, 1984).
- Goldstein, J. L. & Brown, M. S. *Am. J. med.* **58**, 147-150 (1975).
- Pauli, R. M. *Am. J. med. Genet.* **16**, 455-458 (1983).
- Allison, A. C. & Blumberg, B. S. *Am. J. med.* **25**, 933-941 (1958).
- Hirschhorn, K. *Am. J. med. Genet.* **18**, 541 (1984).
- Gusella, J. F. *et al. Nature* **306**, 234-238 (1983).
- Gusella, J. F. *et al. Nature* **318**, 75-78 (1985).
- Gusella, J. F. *et al. Science* **225**, 1320-1326 (1984).

- Gusella, J. F. *et al. Am. J. hum. Genet.* **36**, 139 (1984).
- Negrette, A. *Corea de Huntington*, 2nd edn (La Editorial Universitaria, La Universidad del Zulia, Maracaibo, Venezuela, 1962).
- Avila-Giron, R. in *Advances in Neurology* Vol. 1: *Huntington's Chorea 1872-1972* (eds Barbeau, A., Chase, T. N. & Paulson, G. W.) 261-266 (Raven, New York, 1973).
- Wexler, N. S. *et al. Cytogenet. Cell Genet.* **37**, 605 (1984).
- Wexler, N. S. & Wilentz, J. *Report: Commission for the Control of Huntington's Disease and its Consequences*, DHEW Pub. Nos. NIH 78-1501 & 78-1502 Vol. I, 26-27 & Vol. II, 111-119 (1978).
- Young, A. B. *et al. Neurology* **36**, 244-249 (1986).
- Shoulson, I. *Neurology* **31**, 1333-1335 (1981).
- Folstein, M. F., Folstein, S. E. & McHugh, P. R. *J. Psychiat. Res.* **12**, 189-198 (1975).
- Fisher, J., Kennedy, J., Caine, E. & Shoulson, I. in *The Dementias* (eds Mayeux, R. & Rosen, W.) 229-238 (Raven, New York, 1983).
- Wexler, N. S. in *Advances in Neurology*, Vol. 23: *Huntington's Disease* (eds Chase, T. N., Wexler, N. S. & Barbeau, A.) 257-271 (Raven, New York, 1979).
- Weingartner, H., Caine, E. D. & Ebert, M. H. *J. abnorm. Psychol.* **88**, 52-58 (1979).
- Joyn, R. J. & Shoulson, I. in *Clinical Neuropsychology* (eds Heilman, K. M. & Valenstein, E.) 475-502 (Oxford University Press, New York, 1979).
- McHugh, P. R. & Folstein, M. F. in *Psychiatric Aspects of Neurologic Disease* (eds Benson, D. F. & Blumer, D.) 267-286 (Grune & Stratton, New York, 1975).
- Folstein, S. E., Franz, M. L., Jensen, B. A., Chase, G. A. & Folstein, M. F. *Psychol. Med.* **13**, 45-52 (1983).
- Caine, E. D. & Shoulson, I. *Am. J. Psychiat.* **140**, 728-733 (1983).
- Edwards, J. A. & Gale, R. P. *Am. J. hum. Genet.* **24**, 464-474 (1972).
- Pauli, R. M. *et al. Am. J. med. Genet.* **16**, 459-473 (1983).
- Chemke, J. *et al. J. med. Genet.* **21**, 173-177 (1984).
- Hodgson, S. V. & Saunders, K. E. *J. Med. Genet.* **17**, 478-480 (1980).
- Nordstrom, S. & Thorburn, W. *Clin. Genet.* **18**, 211-216 (1980).
- White, R. A. H. & Akam, M. E. *Nature* **318**, 567-569 (1985).
- Bender, W. *et al. Science* **221**, 23-29 (1983).
- Lewis, E. B., *Am. Zoologist* **3**, 33-56 (1963).
- Shoulson, I. *et al. Neurology* **36** (suppl. 1), 176 (1985).
- Shoulson, I. & Fahn, S. *Neurology* **29**, 1-3 (1979).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137**, 266-267 (1983).
- Anderson, M. A. & Gusella, J. F. *In Vitro* **20**, 856-858 (1984).
- Gusella, J. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 5239-5243 (1979).

Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors

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In early embryonic development the basic body plan arises because cells in different regions become programmed to follow different developmental pathways. We have proposed that in the early amphibian embryo this process of regional specification arises from the action of three different inducing factors, or morphogens (refs 1-5 and Fig. 1), but we have not until now had any idea of their chemical nature. In this paper we report (1) that pure basic fibroblast growth factor (bFGF), at very low concentrations and with high specificity, closely mimics the effect of the ventrovegetal (VV) signal and (2) that the transmission of the natural VV signal can be blocked by heparin, suggesting that it may be a heparin-binding factor such as bFGF.

In the main series of experiments, explants of ectoderm were cut from the animal pole region of stage 8 *Xenopus* blastulae (about 1,024-2,048 cell stage⁶) and exposed to bFGF. At stage 8 the explants include about 50-100 cells, but the cell number continues to increase by cleavage divisions without growth. Control explants round up over about two hours to form spheres with the original pigmented surface exterior and the original blastocoel surface reduced to a small 'yolk plug'. Over the next two days, they become wrinkled and form epidermis around the outer surface. The internal cells may also all become epidermal but often some remain undifferentiated (Fig. 2b, d)^{7,8}. Mesodermal cells are formed rarely, even then probably only because of occasional dissection errors which lead to inclusion of endodermal cells from the blastocoel floor. Treated explants also round up during the first two hours. At about 6 hours, when

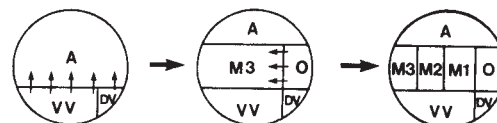


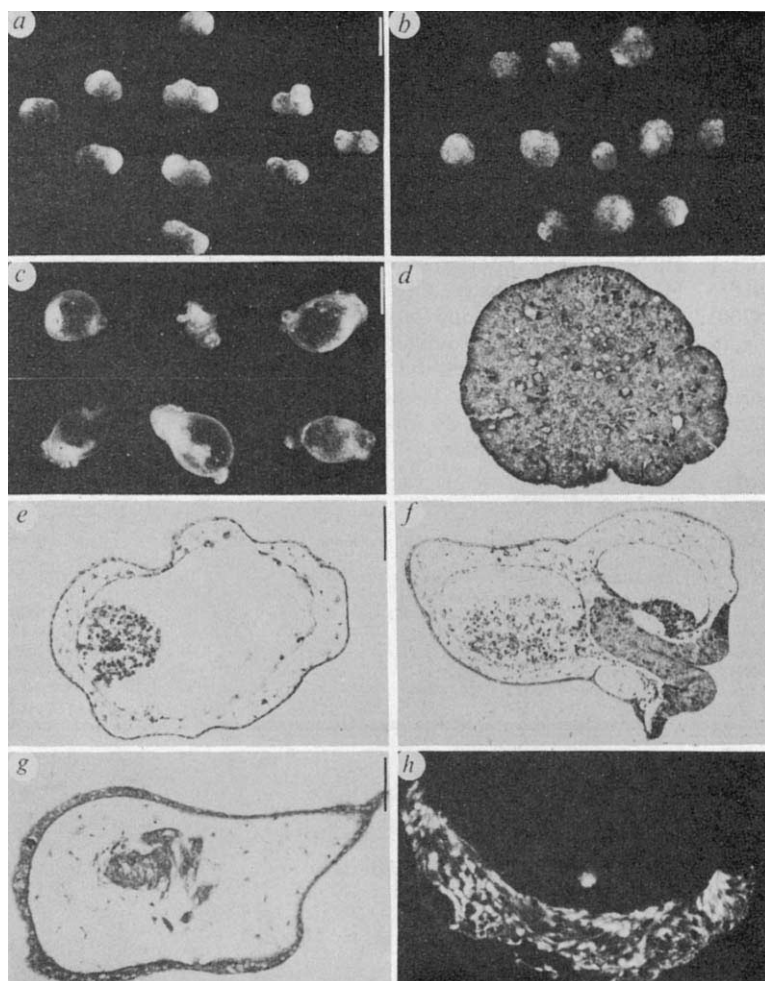
Fig. 1 Model of inductive interactions in the early amphibian embryo based on refs 1-5. The diagram depicts a blastula with the dorsal side to the right. Two mesoderm-inducing signals (DV and VV) are emitted from the vegetal hemisphere and induce an annulus of mesoderm consisting of a small organizer zone (O) and a large ventral mesoderm zone (M3). A dorsaling signal emitted by the organizer then induces intermediate structures such as somites and kidney (M1, M2) from the ventral mesoderm zone. Abbreviations: A, animal hemisphere ('ectoderm'); DV, dorsovegetal; VV, ventro-vegetal; O, organizer; M, mesoderm.

control embryos are middle gastrulae (stage 11-11½), they elongate with the original yolk plug at one end. The degree of elongation varies between batches of embryos and can range from a slight pear-shape departure from sphericity, to a sausage shape about twice as long as it is wide (Fig. 2a). The process is reminiscent of the normal gastrulation movements on the ventral side of the embryo. After about 24 hours, when control embryos are at tailbud stages, the treated explants begin to swell by fluid uptake and become vesicles several times their original volume (Fig. 2c). Specimens were fixed for histological or immunohistochemical examination at about 64 hours of culture (control stage 41).

The appearance depends on the concentration of bFGF used. Between about 2 and 30 ng ml⁻¹ the inductions closely resembled ventral-type mesoderms formed by explants from ventral or lateral marginal zones or by ventrovegetal-animal combinations^{1,2,4,5}. They consist of a concentric arrangement of loose mesenchyme, mesothelium and blood cells within an epidermal jacket and sometimes contain a few muscle cells (Fig. 2e, f). The blood cells do not usually differentiate sufficiently to synthesize haemoglobin but have a characteristic morphological appearance. Between 30 and 120 ng ml⁻¹, most of the explants contain some muscle and significant blocks of it are often produced (Fig. 2g). Their identity was confirmed by

Fig. 2 Effect of bFGF on the development of ectodermal explants. Microoperative, histological and immunohistochemical methods as ref. 2. For stage series see ref. 6. *a*, Some bFGF-treated explants after overnight culture (control stage 20), showing elongation. *b*, Control explants after overnight culture. *c*, Some bFGF-treated explants after 3-day culture, showing vesicular forms. *d*, Section through 3-day control explant, which consists entirely of a solid mass of epidermis. *e*, Section through a bFGF induction, showing a concentric arrangement of epidermis, loose mesenchyme, mesothelium and blood cells. *f*, Section through ventrolateral explant from the marginal zone. These normally form ventral type mesoderm, and include also a region of yolky endodermal cells. *g*, Induction containing a significant muscle block, which arose from treatment with 120 ng ml^{-1} bFGF. *h*, Immunostaining of a muscle-containing induction with antibody to *Xenopus* myosin. Scale bars: *a-c*, $500 \mu\text{m}$; *d-g*, $100 \mu\text{m}$; *h*, $50 \mu\text{m}$. Culture period for *c-h* was 3 days (control stage 41).

Methods. The bFGF used was purified from bovine brain by the method of Gospodarowicz and co-workers^{22,23}, followed by cation exchange chromatography on a Mono S column¹⁷. The mitogenic and mesoderm-inducing activities eluted together, associated with a broad OD peak between 0.4 and 0.5 M NaCl. The material from this peak was characterized as bFGF by the following methods. (1) On SDS-gel electrophoresis it appeared as a doublet with apparent relative molecular masses 16,500 and 18,000²². (2) On C3 reverse-phase chromatography it gave a major peak eluting at 30% acetonitrile which contained all the biological activity¹⁷. (3) Analysis of amino-acid composition of this peak gave values closely resembling those published for bFGF^{22,23}. (4) Activity of a 60 ng ml^{-1} solution was blocked by a 1/240 dilution of an antibody prepared against a synthetic peptide representing the N-terminal 24 residues of bFGF²⁴, but was unaffected by various control sera. The Mono S fraction was not quite pure in that two minor peaks were revealed by the reverse-phase analysis. However, these had divergent amino-acid compositions and were without biological activity. The Mono S material was used for the biological experiments reported below as exposure to acid conditions on the reverse phase column causes a substantial reduction in specific activity. The bFGF concentrations quoted are based on the amino-acid composition analysis.



specific immunofluorescence with an antibody to *Xenopus* myosin (Fig. 2*h*). In general these muscle blocks are smaller than those found in explants from dorsolateral regions of the marginal zone, or in ventral marginal explants dorsalized by co-culture with organizer tissue^{1,4,5}. The ventrolateral character of bFGF inductions provides strong support for our contention that there is a ventral inducing (VV) signal in the embryo, distinct from the organizer-inducing signal (DV) from the dorsovegetal quadrant^{2,5}.

A number of other growth factors were also tested for mesoderm-inducing activity using concentrations ranging from below physiological to 100 times physiological. No activity was found from any of the following: epidermal growth factor, platelet-derived growth factor (PDGF), insulin, chorionic gonadotrophin, interleukin-1 α and β , interleukin-3, interferon α and γ , tumour necrosis factor, transforming growth factor β , or the colony stimulating factors G-CSF and GM-CSF. No activity was found in various commercially available tissue culture media (with or without 10% fetal bovine serum), or in human urine. Activity was found for one other factor: embryonic carcinoma derived growth factor (ECDGF). This has been purified from the culture medium of PC13 murine embryonal carcinoma cells and is quite similar to bFGF in size and in heparin binding although it exhibits a slightly different biological specificity in terms of the spectrum of sensitive target cell types⁹. We have carried out fewer studies on it, but ECDGF appears similar to bFGF in its inductive effects, giving ventral inductions down to $\sim 2 \text{ ng ml}^{-1}$ and some muscle blocks at high concentrations $\sim 100 \text{ ng ml}^{-1}$. Although we cannot prove that there are

no other substances with similar activity the effect does seem to be fairly specific.

We also studied the concentration and time dependence of bFGF action. Concentration dependence was systematically investigated by exposing ectoderm explants to serial twofold dilutions (Fig. 3*a*). Individual explants show an all-or-none response although probably less than half the cells become mesodermal, so the percentage of explants forming vesicles at each concentration is shown. For most growth factors acting on cultured cells there is a range of partial responses over a concentration span of ~ 100 -fold and assays are often based on a linear approximation to this partial response. The induction of mesoderm however follows a rather steeper dose-response curve with a less than 8-fold concentration range of partial responses. Whether this represents a genuine discontinuous (or threshold) response, as predicted for morphogen action¹⁰ or whether it is simply a conventional but steep response is not clear at present. There are some sporadic inductions at the very low concentrations and the 50% response is found at about 2 ng ml^{-1} which is in the reported physiological range for mitogenic action of bFGF on fibroblasts¹¹. Our own preparation was active down to about 50 pg ml^{-1} as a mitogen for murine $10\text{T}\frac{1}{2}$ cells.

Several attempts were made to find the minimum necessary exposure time to obtain a response. Results were variable but suggested that whereas exposures of a few minutes were insufficient, ones of a few hours were sufficient (Fig. 3*b*). This agrees quite well with the estimate made by Gurdon *et al.*¹² that a minimum contact time of about 2.5 h between animal and vegetal explants is required to obtain an induction. The time

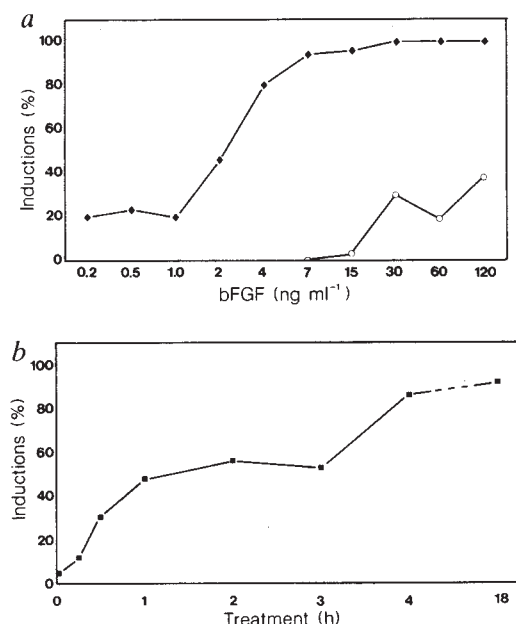


Fig. 3 *a*, Dose-response curve for induction by bFGF. Explants were cultured in Terasaki plates in a final volume of 12 μ l of half strength Normal Amphibian Medium (NAM)² plus 1 mg ml⁻¹ bovine serum albumin (BSA), over 2 μ l of agarose to prevent adhesion to the plastic. Various concentrations of bFGF were present continuously in the wells. Each point represents tests on about 30 explants. Diamonds, percentage of induced explants scored visually as large vesicles; circles, those which proved after sectioning to contain significant muscle blocks such as those in Fig. 2g, *h*. *b*, Effect of withdrawing bFGF at various times. Design as in *a*, except that explants were exposed to bFGF at suprathreshold concentration for various times then removed from the wells, rinsed, and replaced in fresh wells with NAM/2 and 1 mg ml⁻¹ BSA. The total number of explants used was 199.

requirement is also reminiscent of that for induction of DNA synthesis by PDGF^{13,14}. Like PDGF, bFGF leads to the activation of protein kinase C¹⁵, but treatments of ectoderm explants with cAMP, cGMP, A23187 and phorbol 12-myristate-13-acetate, which might be expected to mimic the various second message effects, have so far all proved to have no effect.

The best known effect of bFGF on cultured cells is to stimulate entry into S phase, so we were interested in whether there was any stimulation of ³H-thymidine incorporation in treated explants. Incorporation into batches of explants, treated or untreated, was measured for three two-hour periods between stage 8 and 12, by which time elongation has begun. No stimulation of incorporation was observed and we conclude that the inductive effect is not secondary to growth stimulation. In many respects this is not surprising because cell division is synchronous in *Xenopus* embryos and occurs every 30 min at 25 °C up to the mid-blastula transition (~4,096 cells)¹⁶, and although synchrony breaks down after this there are no systematic regional differences in cell division until after tissues have begun to differentiate during neurulation.

Both bFGF and ECDGF are members of a family of growth factors that bind tightly and specifically to heparin¹⁷. Heparin has been reported to inhibit the binding of bFGF to its receptor¹⁸ and we have found that addition of heparin reduces the effectiveness of the bFGF as an inductor. As the heparin concentration is increased in the presence of a high concentration of bFGF, the production of muscle is lost (Fig. 4) and then the induction is suppressed altogether. This repression occurs at substantial molar excesses of heparin, so it seems likely that the mechanism involves a competition for the bFGF between the heparin in the bulk solution and binding sites within the cell mass. It is not

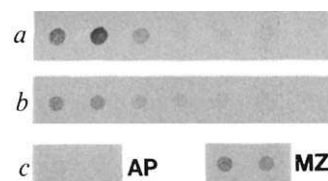


Fig. 4 Detection of muscle in FGF inductions by a dot blot method, using the same antibody to *Xenopus* myosin as in Fig. 2h. *a*, Explants treated with twofold dilutions of bFGF starting at 60 ng ml⁻¹. The first four became vesicles and the first three are positive for muscle. *b*, Explants all treated with 60 ng ml⁻¹ bFGF but in the presence of heparin at concentrations of 0, 0.01, 0.1, 1, 10, 100 and 1,000 μ g ml⁻¹. The first five explants formed vesicles and the blots show the progressive inhibition of muscle formation. *c*, Positive and negative controls are, respectively, marginal zone (MZ) and ectoderm (AP) explants cultured for 3 days.

Methods. After culture, explants were homogenized in 0.12 ml, 50 mM, TrisCl pH 7.4, 1 mM EDTA, 1% sodium deoxycholate, 1 mM phenylmethylsulphonylfluoride (PMSF), 100 nM pepstatin A. This was centrifuged for 4 min at 8,700g to remove pigment granules. The samples were applied to nitrocellulose membrane using a Biorad dot blot apparatus. The membrane was removed and treated as follows. (1) With 3% BSA+5% goat serum in Dulbecco's phosphate-buffered saline A (PBSA), 1 h 37 °C (or overnight 4 °C). (2) Anti-myosin serum 1/200 in PBSA, 2 h room temperature. (3) Several washes in PBSA over 45 min. (4) Add 1/1,000 goat anti-rabbit, affinity-purified horseradish peroxidase (HRP) conjugate (Miles) in 3% BSA 1% goat serum in PBSA, 2 h room temperature. (5) Washed as before and rinsed in water. (6) Add 0.5 mg ml⁻¹ diaminobenzidine, 0.025% cobalt chloride, 0.02% ammonium nickel sulphate, 0.13% H₂O₂ (100 vol.) for about 1 min then rinsed several times in water.

Table 1 Blockage of endogenous mesoderm-including signal by heparin

Combination	Conditions	Positive	Total
AP-VP	No additives	11	14
AP-AP	No additives	0	12
AP-VP	Heparin (1,000 μ g ml ⁻¹)	1	7
AP-VP	Heparin (100 μ g ml ⁻¹)	0	11
AP-VP	Heparin (10 μ g ml ⁻¹)	5	7
AP-VP	Chondroitin sulphate (100 μ g ml ⁻¹)	11	12
AP-VP	No gap, Heparin (1,000 μ g ml ⁻¹)	8	10

AP, animal pole explant; VP, vegetal pole explant. Explants were left in the transfilter apparatus overnight (~16 h) then removed and cultured separately for two more days. Inductions were scored visually by the formation of large vesicles, and were then sectioned. All vesicles indeed contained mesoderm, 75% of a ventral type and the remainder dorsal or intermediate types⁵. The no gap group were combinations assembled in the presence of heparin in an agar-coated dish. Heparin was grade I or grade II from Sigma; chondroitin sulphate from Seikagaku Kogyo Co. Ltd.

necessary to assume that the heparin-bFGF complex is itself inactive.

This effect suggested a way of finding whether the endogenous factor(s) are, like bFGF and ECDGF, heparin-binding factors. It is known that mesoderm inductions, mainly of a ventral character, can be obtained if animal and vegetal explants are cultured on either side of an 0.4- μ m Nucleopore filter¹⁹, showing that the effect is due to diffusible factor(s). A sufficiently large concentration of heparin in the space between the explants might intercept the signal. Transfilter experiments were set up with the explants separated by a nylon gauze about 0.1 mm thick with holes about 0.1 mm square instead of by a Nucleopore filter. This holds the explants apart and allows access of reagents from the side.

The results are shown in Table 1. Controls in which there

were no additives, or in which another glycosaminoglycan, chondroitin sulphate, was included, gave a high frequency of positive inductions. However with heparin at $\geq 100 \mu\text{g ml}^{-1}$ there was only a single positive. In separate experiments it was shown that heparin at 1 mg ml^{-1} has no toxic effects, and no inductive effects on isolated ectoderms. It does not inhibit the formation of mesoderm in animal-vegetal combinations or in marginal zone explants, showing that its presence in solution between the inducing and responding tissues is necessary for blocking.

We cannot, of course, exclude other possible reasons for the blocking action of heparin. But in view of the various controls we feel that the most likely explanation is that the natural ventral mesoderm-inducing factor, in the embryo, is a heparin-binding factor, and that the heparin in the gap can intercept it and prevent it from reaching the target tissue.

In addition to these specific results this work suggests two general conclusions. First, mesoderm induction is a highly specific process, unleashed by a small group of regulatory proteins. This is in sharp contrast to neural induction which can be evoked by a wide range of substances^{20,21}. Second, it suggests a new type of role for growth factors in early development: not as mitogens but as morphogens that control the developmental pathways selected by cells.

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Note added in proof: A third heparin-binding growth factor, acidic FGF (provided by Dr D. Gospodarowicz), is also active in inducing mesoderm.

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1. Smith, J. C., Dale, L. & Slack, J. M. W. *J. Embryol. exp. Morph.* **89**, suppl., 317-331 (1985).
2. Dale, L., Smith, J. C. & Slack, J. M. W. *J. Embryol. exp. Morph.* **89**, 289-313 (1985).
3. Smith, J. C. & Slack, J. M. W. *J. Embryol. exp. Morph.* **78**, 299-317 (1983).
4. Slack, J. M. W. & Forman, D. *J. Embryol. exp. Morph.* **56**, 283-299 (1980).
5. Dale, L. & Slack, J. M. W. *Development* (submitted).
6. Nieuwkoop, P. D. & Faber, J. *Normal table of Xenopus laevis* (N. Holland, Amsterdam, 1967).
7. Slack, J. M. W. *J. Embryol. exp. Morph.* **80**, 321-330 (1984).
8. Slack, J. M. W. *Cell* **41**, 237-247 (1985).
9. Heath, J. K. & Isacke, C. M. *EMBO J.* **3**, 2957-2962 (1984).
10. Lewis, J. H., Slack, J. M. W. & Wolpert, L. *J. theor. Biol.* **65**, 579-590 (1977).
11. Gospodarowicz, D. & Moran, J. S. *J. Cell Biol.* **66**, 451-456 (1975).
12. Gurdon, J. B., Fairman, S., Mohun, T. J. & Brennan, S. *Cell* **41**, 913-922 (1985).
13. Pledger, W. J., Stiles, C. D., Antoniadis, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481-4485 (1977).
14. Singh, J. P., Chaikin, M. A., Pledger, W. J., Scher, C. D. & Stiles, C. D. *J. Cell Biol.* **96**, 1497-1502 (1983).
15. Tsuda, T., Kaibuchi, K., Kawahara, Y., Fukuzaki, H. & Takai, Y. *FEBS Lett.* **187**, 43-46 (1985).
16. Newport, J. & Kirschner, M. *Cell* **30**, 675-686 (1982).
17. Lobb, R. R., Harper, J. W. & Fitt, J. W. *Analyt. Biochem.* **154**, 1-14 (1986).
18. Neufeld, G. & Gospodarowicz, D. *J. biol. Chem.* **260**, 13860-13868 (1985).
19. Grunz, H. & Tacke, L. *Wilhelm Roux Arch. dev. Biol.* **195**, 467-473 (1986).
20. Needham, J. *Biochemistry and Morphogenesis* (Cambridge University Press, 1942).
21. Brachet, J. *Chemical Embryology* (Interscience, New York, 1950).
22. Gospodarowicz, D., Cheng, J., Lui, G. M., Baird, A. & Bohlen, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6963-6967 (1984).
23. Esch, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 6507-6511 (1985).
24. Baird, A. *et al. Recent Prog. Horm. Res.* **42**, 143-205 (1986).

Suppression of *in vitro* haematopoiesis following human immunodeficiency virus infection

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Viral infections are frequently associated with haematological disorders (reviewed in ref. 1). Abnormalities including leukopenia, anaemia and thrombocytopenia are commonly observed in patients with the acquired immune deficiency syndrome (AIDS) or the AIDS-related complex (ARC)²⁻¹². The underlying cause of these haematological abnormalities is poorly understood. We report here that bone marrow progenitors isolated from AIDS or ARC patients are responsive to recombinant human granulocyte-macrophage colony stimulating factor (rGM-CSF) and recombinant erythropoietin. Antibodies present in the serum of patients infected with the human immunodeficiency virus (HIV), however, could suppress the growth of these progenitors, but not the growth of progenitors from HIV seronegative controls. A component of this immune-mediated suppression appears to be antibodies directed towards the envelope glycoprotein (gp120) of HIV.

To study the effects of rGM-CSF and erythropoietin on human progenitor cells derived from patients with AIDS or ARC, disorders due to infection with HIV^{13,14}, adherence-depleted bone marrow mononuclear cells from untreated AIDS and ARC patients as well as healthy, HIV seronegative donors were cultured *in vitro*. At various concentrations of GM-CSF, the number, rate of development, size and morphology of the

granulocyte-macrophage (CFU-GM), early erythroid (BFU-E), or multi-lineage (CFU-Mix) colonies derived from AIDS or ARC bone marrow generally did not differ from that of healthy HIV seronegative donors in the presence or absence of HIV seronegative serum (Fig. 1). In the presence of sera from HIV seropositive patients, however, CFU-GM and BFU-E development was markedly suppressed in bone marrow progenitor cultures from patients with AIDS or ARC, but not in marrow cultures from healthy, HIV seronegative donors (Fig. 1). The suppression by the HIV seropositive serum samples tested ranged from 50 to 90% inhibition in the numbers of CFU-GM or BFU-E colonies observed using different AIDS or ARC bone marrow donors. Suppression was seen in the cultures of bone marrow cells from all the eight AIDS or ARC donors tested but not in any of the cultures of marrow cells from normal donors. Because of the low numbers of CFU-Mix normally observed in cultures established from bone marrow cells from healthy donors or AIDS or ARC patients, suppression of CFU-Mix could not be demonstrated by the addition of HIV seropositive sera.

To investigate further the suppressive effect of serum on CFU-GM and BFU-E colonies derived from AIDS or ARC bone marrow, serum samples obtained from patients before and after they had become positive for HIV antibody were tested for their ability to inhibit AIDS or ARC bone marrow colony formation. There was no effect of the preimmune serum on CFU-GM or BFU-E colony formation, but upon seroconversion the serum markedly suppressed *in vitro* myelopoiesis and erythropoiesis (Fig. 2). This finding was observed in all three AIDS or ARC bone marrows tested. Bone marrows taken from healthy, HIV seronegative donors did not demonstrate any suppression with either the preimmune or HIV seropositive serum (data not shown). Nonspecific effects of serum on allogenic cells (such as, for example, anti-HLA antibodies, anti-blood group antibodies) were not apparent in our study as normal sera did not have any suppressive effect on bone marrow progenitors from individuals with differing ABO blood types. Similarly, preimmune sera did not suppress CFU-GM or BFU-E colony formation in AIDS or ARC bone marrow progenitor cultures but the

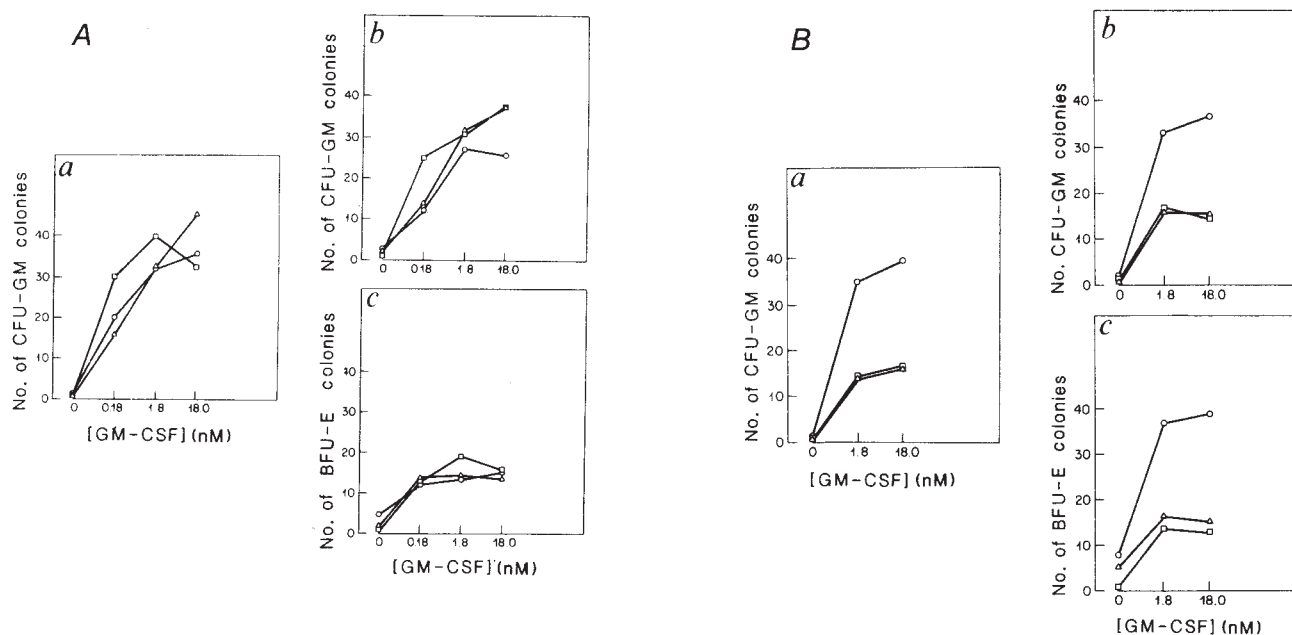


Fig. 1 Effect of sera from HIV-infected patients on GM-CSF-dependent colony formation by bone marrow progenitors from a healthy, HIV-seronegative donor (A) or an HIV-infected donor (B). Adherence-depleted bone marrow progenitor cells were plated in methylcellulose at the indicated concentrations of rGM-CSF in the presence of serum samples from patients with AIDS (Δ), ARC ($\square$), or from HIV seronegative control subjects ($\circ$). The numbers of granulocyte and/or macrophage (a, b) or erythroid (c) colonies per 5×10^4 cells were scored either in the absence (a) or presence (b, c) of recombinant erythropoietin. Each point is the mean of duplicate plates.

Methods. Bone marrow aspirates were taken, after obtaining informed consent, from healthy volunteers unexposed to HIV or from patients with AIDS or ARC who fulfilled the diagnostic criteria for these disorders established by the Centers for Disease Control and the National Institutes of Health. The AIDS or ARC patients neither had active infections involving opportunistic pathogens nor were receiving any potentially myelosuppressive antibiotics, cytotoxic chemotherapy, or other medications. Pathologically, myelodysplasia and erythrodysplasia with maturational abnormalities were noted in the bone marrow biopsies of the AIDS or ARC patients. The bone marrow was aspirated from the posterior iliac crest using standard techniques. The marrow was aspirated into preservative-free heparin (Sigma) and low-density, adherent-depleted cells were obtained by methods previously described³⁷. *In vitro* culture techniques for myeloid and erythroid progenitor cells were as described³⁷. Human sera were added to appropriate plates at a final concentration of 5% (v/v). Sera were tested for HIV antibodies by an enzyme-linked immunosorbent assay (Electro-Nucleonics Inc). The rGM-CSF expressed by CHO cells was purified as described^{38,39} and diluted with Iscove's Modified Dulbecco's Medium (IMDM) so that the protein concentration was $50 \mu\text{g ml}^{-1}$. This solution was diluted further in IMDM so that the final dilution in the culture ranged from $1:10^2$ to $1:10^5$ (18–0.018 nM). In those cultures which required the addition of erythropoietin, one unit of highly purified recombinant erythropoietin⁴⁰ derived from CHO cells was added dropwise to each of the cultures three days after the start of incubation. Granulocyte and/or macrophage (CFU-GM), early erythroid (BFU-E), and mixed-lineage (CFU-Mix) colonies were scored on day 12–14. Four additional normal bone marrows and seven other AIDS or ARC bone marrows tested showed similar results to the one presented here.

post-immune, HIV seropositive sera did. The myelosuppressive effect was, therefore, only seen in the HIV seropositive serum samples on bone marrow from AIDS or ARC patients.

In preliminary studies, we have been able to isolate HIV from pooled bone marrow progenitor colonies grown *in vitro* from an AIDS patient and were able to infect adherence-depleted bone marrow taken from a HIV seronegative donor (Table 1). Virus, however, could not be isolated from the pooled progenitors of three other AIDS patients. This failure may be explained by the observation that GM-CSF can inhibit HIV production from cells of myeloid origin, such as has been shown for the monocyte cell line U937¹⁵, or explained by the variability of HIV recovery from infected hosts using available culture techniques. These observations suggest that progenitors and/or terminally differentiated cells in the cultures may be infected with HIV. HIV infection of the host may not be directly cytopathic to bone marrow progenitors because, in the absence of anti-HIV antibodies, progenitor proliferation occurs normally *in vitro* in the presence of purified rGM-CSF (Fig. 1). Still other progenitor cells may be refractory to infection because, even in the presence of HIV seropositive serum, colony formation is not completely inhibited. Recently, we (J. Sullivan, J.E.G. *et al.*, unpublished observations), as well as others¹⁶, could detect virus infection of myeloid precursors in bone marrow from AIDS patients by *in situ* hybridization. Similarly, follicular dendritic cells and monocyte-macrophages, the progeny of CFU-GM or CFU-M, have been shown to be infected with HIV *in vivo*^{17–23}

and monocyte-macrophages and established cell lines of human B cells, monocytes, myeloblasts and promyelocytes could be infected with HIV *in vitro*^{15,21–26}. These cells do not demonstrate gross cytopathic effects or impaired proliferation.

One hypothesis to explain the suppressive effects of sera is that bone marrow progenitor cells are infected by HIV and those infected cells may have HIV antigens such as the major envelope glycoprotein, gp120, or the transmembrane protein, p41, expressed on the cell surface. Antibodies to retroviral antigens might inhibit the proliferation of the virally infected progenitor cells directly or indirectly through an antibody/dependent mechanism such as antibody-dependent cell-mediated cytotoxicity (ADCC). To test directly whether the suppressive activity found in sera from HIV-infected patients is due to anti-HIV antibody, immunoglobulin from a patient with AIDS was purified and added at several concentrations to bone marrow progenitors from an ARC patient (Fig. 3). When compared to purified pooled control immunoglobulin, the purified AIDS immunoglobulin showed significant suppression of haematopoietic colony formation. These observations have been extended in experiments using rabbit heteroantiserum directed against recombinant gp130 which has previously been shown to neutralize HTLV-3b infection of H9 cells²⁷. Using the rabbit's preimmune serum and heteroantiserum against recombinant gp130 at a final concentration of $12.5 \mu\text{g ml}^{-1}$, we found in duplicate cultures a decrease in CFU-GM from a mean of 18.5 colonies per 5×10^4 cells to a mean of 8.5 colonies per 5×10^4 cells. This observation suggests

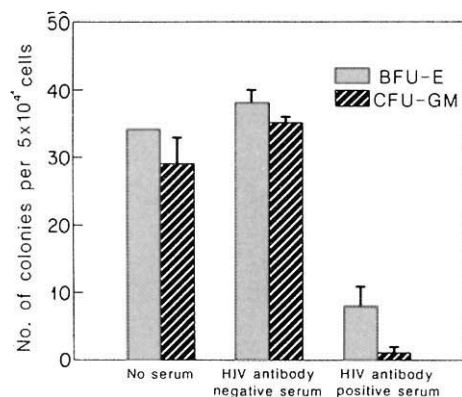


Fig. 2 Appearance of haematopoietic-suppressive activity in serum from a patient following HIV seroconversion. Adherence-depleted bone marrow progenitor cells from a patient with AIDS were plated in the presence of rGM-CSF (1.8 nM) and recombinant erythropoietin (1 U ml⁻¹) in the presence of serum from a patient before and after apparent infection by HIV. Each individual point represents the mean colony count ($\pm$ s.d.) of duplicate plates. The preimmune serum which was HIV seronegative was drawn more than six months before the patient was found to be HIV seropositive. Culture methods as in Fig. 1. Marrow samples from two additional AIDS or ARC patients have also been tested with these serum samples and both gave results similar to the results presented.

Table 1 Reverse transcriptase activity in bone marrow cultures

	Day 7	Day 11	Day 14	Day 19	Day 21
Bone marrow from AIDS patient (WS)	5.13	5.27	4.09	1.49	0.49
Normal bone marrow infected with control supernatant (+epo)	0.00	0.00	0.00	0.00	0.00
Normal bone marrow infected with HTLV-IIIb (+epo)	0.61	6.44	7.30	6.01	3.16
Normal bone marrow infected with control supernatant (-epo)	0.00	0.00	0.00	0.00	0.00
Normal bone marrow infected with HTLV-IIIb (-epo)	0.00	0.00	0.69	1.08	1.05

Reverse transcriptase activity is given as c.p.m. $\times 10^5$. Bone marrow was grown in culture for 12–14 days as in Fig. 1. The cultures were then pooled, washed in RPMI plus 20% fetal calf serum (FCS) and 0.2% hexadimethrine bromide (Sigma) and co-cultivated with 10^6 peripheral blood mononuclear cells from a HIV seronegative donor in the presence of 20 μ g phytohaemagglutinin (Sigma) and 10% interleukin-2 (Cellular Products). Supernatants were collected at various time intervals and assayed for reverse transcriptase activity as described²⁷. The normal bone marrow was inoculated either with the HTLV-IIIb isolate of HIV or the control supernatant from uninfected H9 cells for 2 h, after which the adherent cells were depleted and the cultures established from the low-density population. The normal bone marrow progenitors were grown in the presence or absence of erythropoietin (epo); the cultures were pooled and co-cultivated for recovery of HIV as for the AIDS bone marrow.

that antibody directed against the major envelope glycoprotein of HIV contributes to the myelosuppression observed *in vitro*. From our initial studies, we suggest that the progenitor cells may be infected with HIV and are not grossly perturbed in their proliferative potential unless antibody to HIV is present.

In other disease conditions associated with peripheral cytopenias, serum is a potent source of a variety of colony-stimulating activities^{28–30}. For example, we have found that sera from many patients with aplastic anaemia can readily support colony formation by normal bone marrow progenitor cells in the absence of additional rGM-CSF (R.E.D., J. M. Rapoport

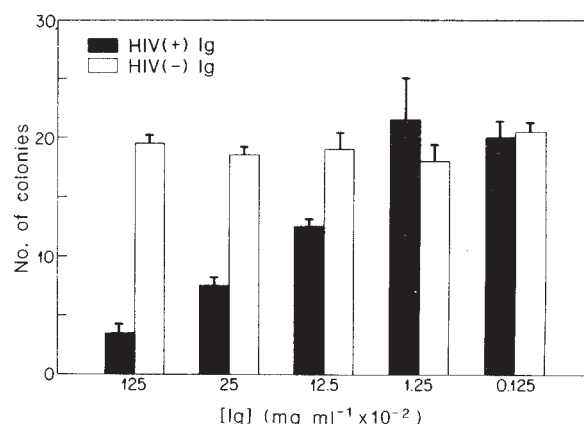


Fig. 3 Inhibition of GM-CSF-dependent colony formation of bone marrow cells from an HIV-infected patient by purified immunoglobulin from an AIDS patient. Adherence-depleted bone marrow progenitor cells from an ARC patient were plated in methylcellulose cultures in the presence of rGM-CSF and the indicated amounts of purified immunoglobulin from an AIDS patient (■) or from a pool of sera from normal donors (□). Each colony count represents the mean ($\pm$ s.d.) of duplicate determinations.

Methods. The immunoglobulin fraction of serum from an AIDS patient was obtained by ammonium sulphate precipitation followed by elution from a protein A-Sepharose (Pharmacia) column as described²⁷. Protein concentration by amino-acid analysis was 25 mg ml⁻¹. The normal human immunoglobulin G (IgG) used (Cappel) was shown to be negative for HIV antibodies by an enzyme-linked immunosorbent assay (Electro-Nucleonics Inc.). The normal human immunoglobulin was resuspended in phosphate-buffered saline to a concentration of 25 mg ml⁻¹. These immunoglobulins were diluted in IMDM to the final concentrations shown. Methods for establishing the cultures were as in Fig. 1, except that recombinant erythropoietin was not added to these cultures. GM-CSF was added at a final concentration of 1.8 nM. No colony growth was observed in the absence of GM-CSF.

and D. G. Nathan, unpublished observations). In contrast, none of the serum samples from either AIDS or ARC patients, despite their haematologic abnormalities, had any colony-stimulating activity as measured in our assay system with normal bone marrow target cells. This implies that there is a defect in haematopoietic growth factor production in HIV-infected patients. Others³¹ have reported defects in lymphokine production by T lymphocytes from AIDS patients which could account for some of the depression of colony-stimulating activity found in the sera from these individuals. Still others have reported the production of soluble suppressor factors from the peripheral blood mononuclear cells of AIDS or ARC patients^{32,33}. It will also be interesting to examine production of haematopoietins by HIV-infected monocytes because these cells are known to produce colony-stimulating activity³⁴ as well as other proteins including interleukin-1³⁵, which has recently been demonstrated to activate the expression of GM-CSF³⁶. The inability of the virally infected T cells and monocytes to produce factors regulating the production of haematopoietic cells in combination with the haematopoietic suppressive effect of antibodies directed against HIV would certainly contribute to the common occurrence of cytopenias in AIDS and ARC patients.

The finding that sera from HIV-infected patients are capable of inhibiting GM-CSF-dependent colony formation by bone marrow progenitor cells in AIDS and ARC patients potentially complicates the use of hematopoietins in AIDS patients. The suppression of GM-CSF-dependent colony formation by the sera of AIDS patients, however, was never complete and the results obtained in an immunodeficient, pancytopenic rhesus monkey (*Macaca mulatta*) naturally infected by a Type D retrovirus³⁷ indicate that administration of this growth factor may be effective in stimulating haematopoiesis in AIDS patients. We

expect that clinical studies of the effectiveness of GM-CSF in overcoming the virus-induced cytopaenias in AIDS patients as well as further characterization of the HIV-induced haematopoietic suppressive activity to provide insight into the pathophysiology of haematopoiesis following retroviral infection in man.

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1. Young, N. & Mortimer, P. *Blood* **63**, 729-737 (1984).
2. Abrams, D. I. *et al. Am. J. clin. Path.* **81**, 13-18 (1984).
3. Spivak, J. L., Bender, B. S. & Quinn, T. C. *Am. J. Med.* **77**, 224-228 (1984).
4. Schneider, D. R. & Picker, L. J. *Am. J. clin. Path.* **84**, 144-152 (1985).
5. Castella, A., Croxson, T. S., Mildvan, D., Witt, D. H. & Zalunsky, R. *Am. J. clin. Path.* **84**, 425-432 (1985).
6. Geller, S. A., Muller, R., Greenberg, M. L. & Siegal, F. P. *Archs Path.* **109**, 138-141 (1985).
7. Zon, L. I., Arkin, C. & Groopman, J. E. *Br. J. Haemat.* (in the press).
8. Morris, L. *et al. Ann. Intern. Med.* **96**, 714-717 (1982).
9. Walsh, C., Nardi, M. & Karparkin, S. *New Engl. J. Med.* **311**, 635-639 (1984).
10. Stricker, R. B., Abrams, D. I., Corash, L. & Shuman, M. A. *N. Engl. J. Med.* **313**, 1375-1380 (1985).
11. Murphy, M. F. *et al. Lancet* **i**, 217-218 (1985).
12. Leiderman, I. Z., Greenberg, M. L., Adelsberg, B. R. & Siegal, F. P. in *AIDS* (eds Gottlieb, M. S. & Groopman, J. E.) 281-289 (Liss, New York, 1984).
13. Barre-Sinoussi, F. *et al. Science* **220**, 868-871 (1983).
14. Gallo, R. C. *et al. Science* **224**, 500-503 (1984).
15. Hammer, S. N., Gillis, J., Groopman, J. E. & Rose, R. M. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8734-8738 (1986).
16. Busch, M., Beckstead, J., Gantz, D. & Vyas, G. *Blood* **68**, 122a (1986).
17. Armstrong, J. A. & Horne, R. *Lancet* **ii**, 370-372 (1984).
18. Gartner, S. *et al. Science* **233**, 215-219 (1986).
19. Gyorkey, F., Melnick, J. L., Sinkovics, J. G. & Gyorkey, P. *Lancet* **i**, 106 (1985).
20. Koenig, S. *et al. Science* **233**, 1089-1093 (1986).
21. Salahuddin, S. Z., Rose, R. M., Groopman, J. E., Makhani, P. D. & Gallo, R. C. *Blood* **68**, 281-284 (1986).
22. Ho, D. D., Rota, T. R. & Hirsch, M. S. *J. clin. Invest.* **77**, 1712-1715 (1986).
23. Gartner, S. *et al. Science* **233**, 215-219 (1986).
24. Levy, J. A. *et al. Virology* **147**, 441-448 (1985).
25. Nicholson, J. K. A., Cross, G. D., Callaway, C. S. & McDougal, J. S. *J. Immun.* **137**, 323-329 (1986).
26. Cortes, E., Koeffler, H. P., Gaynor, R. & Mitsuyasu, R. *Blood* **68**, 123a (1986).
27. Lasky, L. A. *et al. Science* **233**, 209-212 (1986).
28. Metcalf, D. *Int. J. Cancer* **27**, 577-584 (1981).
29. Hall, B. M. *Br. J. Haemat.* **17**, 553-561 (1969).
30. Morley, A., Quesenberry, P., Bealmeier, P., Stohlman, F. & Wilson, R. *Proc. Soc. exp. Biol. Med.* **140**, 478-480 (1972).
31. Murray, H. W., Rubin, B. Y., Masur, H. & Roberts, R. B. *New Engl. J. Med.* **310**, 883-884 (1984).
32. Cunningham-Rundles, S., Michelis, M. A. & Masur, H. *J. clin. Immun.* **3**, 156-165 (1983).
33. Laurence, J., Gottlieb, A. B. & Kunkel, H. G. *J. clin. Invest.* **72**, 2072-2081 (1983).
34. Chervenick, P. A. & LoBuglio, A. F. *Science* **178**, 164-166 (1972).
35. Dinarello, C. A. *Rev. infect. Dis.* **6**, 51-95 (1984).
36. Zucali, J. R. *et al. J. clin. Invest.* **77**, 1857-1863 (1986).
37. Donahue, R. *et al. Nature* **321**, 872-875 (1986).
38. Wong, G. G. *et al. Science* **228**, 810-815 (1985).
39. Wong, G. G. *et al. in Cancer Cells* Vol. 3 (eds Feramisco, J., Ozzanne, B. & Stiles, C.) 235-241 (Cold Spring Harbor Laboratory, New York, 1985).
40. Jacobs, K. *et al. Nature* **313**, 806-810 (1985).

Localization of clathrin light-chain sequences mediating heavy-chain binding and coated vesicle diversity

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At least four distinct forms of clathrin light chains are found in mammalian cells¹⁻⁵. This molecular variability derives from tissue-specific patterns of expression of LC_a and LC_b genes⁶. Sequence analysis shows an overall homology of 60% between LC_a and LC_b, and the presence of brain-specific insertion sequences. These findings suggest that the different light chains have both shared and specialized functions⁶. To address this question we have used a panel of monoclonal antibodies to identify two structurally and functionally distinct regions in the clathrin light-chain sequences. One region (residues 158-208) is exposed in native clathrin structures (triskelions^{7,8} and coated vesicles) and includes the brain-specific insertion sequences. The second region (residues 93-157), which is cryptic in native clathrin structures, is involved in binding the clathrin heavy chain and contains the region of strongest homology with intermediate filament proteins⁶.

A shared feature of all four light chains is their binding to the clathrin heavy chain. We reasoned that sites involved in

heavy-chain binding should be accessible in free light chains but not in triskelions or coated vesicles. These were identified using a panel of 18 monoclonal antibodies raised against separated light chains. Figure 1 shows the specificity of these antibodies for brain and non-brain forms of LC_a and LC_b in a solid-phase radioimmunoassay and their binding to coated vesicles in a co-sedimentation assay. Eleven of the anti-light-chain antibodies bound as well to coated vesicles as the X22 antibody which recognizes an exposed determinant of the clathrin heavy chain^{9,10}. The other seven antibodies gave weak or no binding to coated vesicles although they react strongly with free light chains. In addition, this second group of antibodies shows no reactivity with purified clathrin triskelions as measured by immunoprecipitation, although they do immunoprecipitate free light chains. In contrast, all but two of the antibodies (LCB.72,74) which bind to coated vesicles are effective in immunoprecipitation of triskelions. Thus light-chain determinants become cryptic as a result of light chain-heavy chain association within the clathrin triskelion rather than from triskelion assembly into coated vesicles.

Seven of the antibodies tested react exclusively with the brain forms of the clathrin light chains. All show preferential binding to LC_b, four are LC_b-specific and three react weakly with LC_a. The specificity of these antibodies suggests they are directed against sites involving the brain-specific insertion sequences. As five of these antibodies react in Western blots, presumably recognizing linear determinants, it is likely that they interact directly with residues in the insertion sequences. This is confirmed by the peptide-binding studies described here. A critical finding is that all seven of these tissue-specific antibodies bind to sites that remain exposed when clathrin light chains are associated with triskelions or coated vesicles. This indicates that the brain-specific sequences are accessible in the *in vivo*, functional forms of clathrin. It is therefore possible that, in the brain, these sequences provide sites of interaction with other proteins involved in brain-specific functions of coated vesicles.

A second method was used to identify light-chain sites involved in interactions with the clathrin heavy chain. This method measures the capacity of monoclonal antibodies to inhibit the

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Antibody	LC specificity				Coated vesicle binding (cpm $\times 10^{-3}$)				
	Brain		Adrenal		10	20	30	40	
	LC _a	LC _b	LC _a	LC _b					
Control	-	-	-	-					
X43	+	+	+	+					
X49	+	+	+	+					
X50	+	+	+	+					
X16	+	-	+	-					
X44	-	+	-	+					
X45	-	+	-	+					
X46	-	+	-	+					
CVC.1	+	-	+	-					
CVC.6	+	-	+	-					
CVC.7	+	-	+	-					
LCB.1	(+)	+	+	+					
LCB.2	(+)	+	-	-					
LCB.4	(+)	+	-	-					
LCB.3	-	+	-	-					
LCB.5	(+)	+	-	-					
LCB.6	-	+	-	-					
LCB.72	-	+	-	-					
LCB.74	-	+	-	-					
X22	-	-	-	-					

Fig. 1 Binding properties of anti-light-chain antibodies. Monoclonal antibodies reacting with purified clathrin light chains were tested for binding to bovine brain coated vesicles by co-sedimentation. The specificity of each antibody for separated LC_a and LC_b light chains (LC) derived from brain and adrenal clathrin is indicated to the left of the vesicle-binding data: +, strong reactivity; (+), weak reactivity; -, no reactivity.

Methods. Monoclonal antibodies were produced and isolated according to established procedures¹⁴ by fusion of mouse popliteal lymphocytes or splenocytes with the SP2/0 cell line¹⁵ and rat popliteal lymphocytes with the YB2/0 cell line¹⁶. Antibodies used in all studies were purified from ascites fluid of mice or rats¹⁷. X43-X50 were derived from rats (Lou/M) immunized with purified LC_b in footpads⁹. LCB.1-LCB.4 were derived from mice (BALB/c) immunized with bovine brain LC_b intraperitoneal and triskelions intravenous¹⁸. LCB.5-LCB.74 were derived from mice (BALB/c) immunized with brain LC_b C.1 peptide (amino acids 158-228) in footpads⁹. Production of CVC.1-CVC.7¹¹, X16 and X22⁹ was as reported. Antibody specificity for purified light chains was determined by indirect solid-phase radioimmunoassay⁹. Antibody binding to light chains, immobilized on a polyvinyl chloride plate, was detected using iodinated F(ab')₂ fragment of rabbit anti-mouse Ig (¹²⁵I-RAM). To obtain clathrin light chains, coated vesicles were isolated from bovine brain or adrenal tissue¹⁹, suspended in 10 mM Tris, 0.2 mM EDTA, pH 7.5 and exposed to 100 °C for 10 min. After centrifugation, LC_a and LC_b were purified from the supernatant by DEAE chromatography². The LC_a pool was depleted of residual LC_b, and the LC_b pool depleted of residual LC_a using affinity columns of X45-Sepharose 4B and X16-Sepharose 4B respectively⁹. Coated vesicles (CV) for cosedimentation were prepared from bovine brains¹⁹ and suspended at 100 mg ml⁻¹ in 100 mM MES, 2.5 mM CaCl₂, 0.5 mM MgCl₂, pH 6.5 (buffer A') with 0.02% NaN₃. To assay antibody binding to coated vesicles, 1 mg of CV was combined with 5 µg purified monoclonal antibody in 100 µl of buffer A' with 0.5% bovine serum albumin (buffer A'-BSA) incubated 1 h, 4 °C then washed twice with 1 ml buffer A'-BSA by centrifugation for 10 min at 13,000g. The ¹²⁵I-RAM (100,000 c.p.m.) in 10 µl was incubated with the CV pellet in 100 µl buffer A'-BSA, 1 h, 4 °C, then washed twice, as before. CV pellets were counted for radioactivity bound: control binding (background) was determined using 5 µg of anti-Leu 10 a monoclonal antibody recognizing a human cell surface antigen. X22 reacts with clathrin heavy chain⁹.

reassociation of separated light chains with heavy chains. Only three light-chain-specific antibodies (X16, X43, X45) gave a significant inhibition of subunit reassociation, and all are directed against sites that become cryptic on association with the heavy chain. Similar inhibition of light chain-heavy chain association was seen with either purified immunoglobulin G (IgG) or monovalent antigen-binding fragments (Fab) frag-

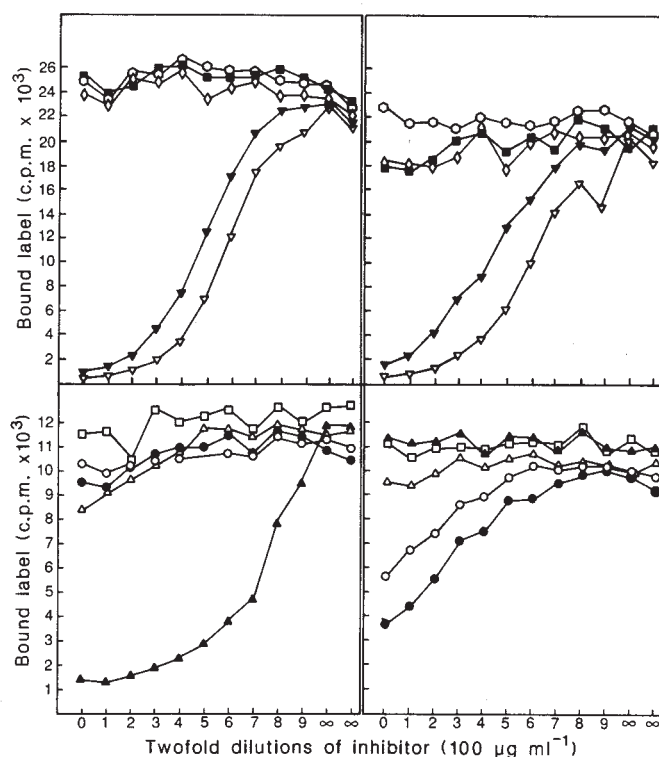


Fig. 2 Inhibition of ¹²⁵I-labelled LC_a (left panels) and ¹²⁵I-labelled LC_b (right panels) binding to clathrin heavy chain with Fab fragments of anti-light-chain antibodies. Iodinated LC_a and LC_b light chains were tested for binding to clathrin heavy chain in the presence of increasing dilutions of Fab fragments produced from anti-light chain antibodies. The two left-hand panels show effects on LC_a binding and the right-hand panels show effects on LC_b binding, of each inhibitor, indicated as follows: ■, LCB.6 Fab; ○, CVC.7 Fab; ◇, X50 Fab; ▽, LC_b; ▴, LC_a; □, anti-Leu 10 Fab; △, X49 Fab; ○, X45 Fab; ●, X43 Fab; ▲, X16 Fab. Unlabelled LC_a and LC_b each inhibit binding of both light chains^{2,3}. Results shown here represent light-chain binding to heavy chain prepared by mild proteolysis of triskelions. Similar results were obtained when heavy chain was prepared by proteolysis of clathrin baskets.

Methods. Iodinated LC_a and LC_b were each tested for binding to clathrin heavy chain in the presence of dilutions of Fab fragments of anti-light chain antibodies or purified LC_a and LC_b, starting at 100 µg ml⁻¹, in a competitive solid-phase RIA⁹. Bovine brain LC_a and LC_b (purified and separated as in Fig. 1) were iodinated with 1 mCi ¹²⁵I-sodium iodide per 25 µg using Enzymobeads (Biorad). 5 × 10⁵ c.p.m. LC_a or 2.5 × 10⁵ c.p.m. LC_b in 20 µl phosphate-buffered saline (PBS), pH 7.4 with 0.5% bovine serum albumin (PBS-BSA) were used per assay. Clathrin heavy chains were prepared by elastase digestion of purified triskelions¹¹ at a 1:12 ratio of enzyme to protein. Heavy chains were applied to polyvinyl chloride plates at 350 µg ml⁻¹ in 0.5 M Tris-HCl, 1 mM EDTA, pH 7.0 and the assay carried out in PBS-BSA. When basket-derived heavy chains were used, the assay was carried out under conditions maintaining basket stability¹⁰. Fab fragments were generated from monoclonal antibodies using established methods²⁰.

ments (Fig. 2) thus ruling out artefactual effects caused by aggregation due to antibody bivalency. Furthermore, inhibitory effects were observed whether light chains were reassociating with heavy chains derived from free or assembled triskelions. The inhibitory effects correlated with the light-chain specificity of the antibodies. Fab fragments of the anti-LC_a antibody X16 strongly inhibited LC_a association with heavy chain but did not affect LC_b association: those from the anti-LC_b antibody X45 had the reverse effect. Fab fragments of X43, which binds more strongly to LC_b than LC_a, had a detectable inhibitory effect only on LC_b binding. Fab fragments of two additional antibodies recognizing cryptic sites (X49 and X50) and of two antibodies

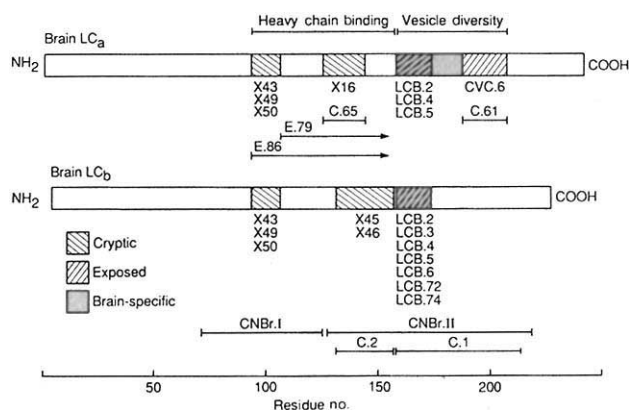


Fig. 3 Structural regions of the clathrin light chains mediating heavy-chain binding and coated vesicle diversity. The location of antibody-binding sites and light-chain peptides in the primary sequences of brain LC_a and LC_b are drawn to scale based on the data in ref. 6. Stipple, brain-specific insertion sequences. The LC_a insertion sequence has 12 additional amino acids compared with the LC_b insertion sequence. Antibody-binding sites were deduced by reactivity with peptides, and the reactive antibody is listed below the sequence containing its binding site (shaded region). Differential shading, sequences which contain exposed or cryptic antibody-binding sites. The protein sequences of peptides produced from clathrin light chains were determined⁶, and the sequence spanned by each peptide is indicated below the map of each light chain from which it was derived.

Methods. Light-chain peptides were produced by digestion with clostripain (C peptides) or elastase (E peptides) or by CNBr cleavage, and fractionated or purified by reverse-phase HPLC⁶. The C.1 peptide extends from either residue 158 or 162 to 288, but is shown as starting with 158. The C.2 peptide extends from 131 to 157, based on relative molecular mass. It was sequenced up to residue 146. The carboxy-terminal amino acids of peptides E.79 and E.86 are not known but the CVC.6 site is included in E.86 but not in E.79. Antibody binding to peptides was measured by indirect solid-phase radioimmunoassay⁹. Aliquots of HPLC fractions containing purified peptides (3 μ l at $\sim 100 \mu\text{g ml}^{-1}$ in 0.1% trifluoroacetic acid) were dried onto wells of polyvinyl chloride plates. After rehydration in PBS, wells were saturated with PBS-BSA, then tested for binding of anti-light chain monoclonal antibodies (50 μ l at $20 \mu\text{g ml}^{-1}$ PBS-BSA or 50 μ l hybridoma culture supernatant). Antibody binding was detected with ¹²⁵I-RAM. Each antibody reacted only with the peptides which are shown as spanning the antibody-binding site on each light chain, demonstrating no cross-contamination of peptides. Peptide-binding reactions were $>10,000$ c.p.m., with backgrounds of <1000 c.p.m.

binding to exposed sites (CVC.7 and LCB.6) had no effect on light chain-heavy chain reassociation. These results show that the cryptic antigenic sites recognized by X16, X45 and X43 are light-chain determinants involved in contacting the clathrin heavy chain, in both the triskelion and coated vesicle structures.

To identify light-chain sequences recognized by the monoclonal antibodies and thus to localize the exposed and cryptic regions, anti-light-chain antibodies were tested for binding to the purified peptides used for primary structure determination⁶. Fourteen of 16 antibodies tested reacted with isolated peptides, as measured by indirect radioimmunoassay (Fig. 3). The binding sites of the X16, CVC.6 and X45/X46 antibodies were determined by reactivity with single peptides C.65, C.61 and C.2, respectively. The X43/X49/X50 binding site was mapped by reactivity with the CNBr.I peptide from LC_b and by differential binding to the E.86 and E.79 peptides which both contain the X16 site of LC_a. The brain-specific antibodies were mapped to the brain LC_b insertion sequence, and the homologous region of the brain LC_a insertion sequence, based on binding to the C.1 peptide of LC_b.

These results define two distinct structural regions of the light chains. The exposed sites on LC_a and LC_b are located in residues 158–208, a region which contains the brain-specific insertion

sequences. The cryptic determinants are localized to a region involving residues 93–157. All the antigenic determinants mapped are found in the hydrophilic sequences which span the carboxy-terminal 60% of each light chain and are characterized by their content of basic amino acids. It is possible that this property accounts for the greater apparent antigenicity of this region. Further examination of hydrophilicity plots of brain light-chain sequences indicates that reduced hydrophilicity at the beginning of the insertion sequences bisects the hydrophilic region in brain LC_a and LC_b (see Fig. 3 of ref. 6). This division corresponds exactly to the division between the cryptic and exposed sites detected by the anti-light-chain antibodies.

Localization of two structurally distinct regions of the clathrin light chains has revealed how different sequences could mediate separate functions. The first region (residues 93–157) is involved in binding the clathrin heavy chain. The sequence is predicted to be α -helical and includes the region of strongest homology (residues 124–149) with intermediate filament proteins⁶. This suggests that light chain-heavy chain interactions may involve contacts between a light-chain α -helix and a helix-binding structure on the heavy chain.

The second region (residues 158–208) is exposed to the cytoplasm and contains the brain-specific insertion sequences and the adjacent sequence recognized by the CVC.6 antibody (residues 188–208) on brain and non-brain LC_a. It has been previously shown that the CVC.6 antibody binds close to the triskelion vertex¹¹, but antibodies against other light chain determinants map further along the proximal part of the arm². These data suggest that the light chain is oriented with its carboxy terminus toward the centre of the triskelion. In addition, this result localizes the CVC.6 binding site and the adjacent brain-specific sequence to a protruding point on the assembled coated vesicle¹². The exposed location of the polymorphic light-chain sequences indicates a role in influencing diversity of coated vesicle function. This influence may be due to light-chain effects on the conformation of the triskelion vertex, resulting in diversity of coated vesicle size and shape^{2,13}. Accessibility of polymorphic sequences also provides a potential recognition site for coated-vesicle binding proteins which could mediate tissue-specific functions.

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- Pearse, B. M. F. *J. molec. Biol.* **126**, 803–312 (1978).
- Ungewickell, E. *EMBO J.* **2**, 1401–1408 (1983).
- Winkler, F. K. & Stanley, K. K. *EMBO J.* **2**, 1393–1400 (1983).
- Creutz, C. E. & Harrison, J. E. *Nature* **308**, 208–210 (1984).
- Holmes, N., Biermann, J. S., Brodsky, F. M., Bharucha, D. & Parham, P. *EMBO J.* **3**, 1621–1627 (1984).
- Jackson, A. P., Seow, H. F., Holmes, N., Drickamer, K. & Parham, P. *Nature* **326**, 154–159 (1987).
- Ungewickell, E. & Branton, D. *Nature* **289**, 420–422 (1981).
- Kirchhausen, T. & Harrison, S. C. *Cell* **23**, 755–761 (1981).
- Brodsky, F. M. *J. Cell Biol.* **101**, 2047–2054 (1985).
- Blank, G. S. & Brodsky, F. M. *EMBO J.* **5**, 2087–2095 (1986).
- Kirchhausen, T., Harrison, S. C., Parham, P. & Brodsky, F. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2481–2485 (1983).
- Vigers, G., Crowther, R. A. & Pearse, B. M. F. *EMBO J.* **5**, 529–534 (1986).
- Pearse, B. M. F. & Bretscher, M. A. *Rev. Biochem.* **50**, 84–101 (1981).
- Galfré, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).
- Shulman, M., Wilde, C. D. & Köhler, G. *Nature* **276**, 169–270 (1978).
- Kilmartin, J. V., Wright, B. & Milstein, C. *J. Cell Biol.* **93**, 576–582 (1982).
- Parham, P., Androlewicz, M. J., Brodsky, F. M., Holmes, N. J. & Ways, J. P. *J. Immun. Meth.* **53**, 133–173 (1982).
- Stähli, C., Staehelin, T., Miggiaro, V., Schmidt, J. & Häring, P. *J. Immun. Meth.* **32**, 297–304 (1980).
- Pearse, B. M. F. & Robinson, M. S. *EMBO J.* **3**, 1951–1957 (1984).
- Parham, P. in *Handbook of Experimental Immunology* Vol. 1 (eds Weir, D. M., Herzenberg, L. A., Blackwell, C. & Herzenberg, C. A.) 14.1–14.23 (Blackwell Scientific, Oxford, 1986).

Old inflation and anisotropy

BARROW and Turner¹ have studied the effects of anisotropy on Guth's original model of inflation². They concluded that anisotropy, if large, could prevent inflation from occurring. Though Guth's 'old inflation' suffers from the fatal 'graceful exit' problem (models which undergo sufficient inflation never again become radiation-dominated³), and has been supplanted by Linde's and Albrecht and Steinhardt's 'new inflation'^{4,5}, the work of Barrow and Turner is still frequently referred to. Further, it has been argued that their conclusions are incorrect, although we find the arguments of these authors to be invalid or inapplicable. For these reasons, we reexamined the paper by Barrow and Turner. In doing so, we have uncovered an error in their paper and find their basic conclusion to be wrong; large amounts of initial anisotropy do not, in general, prevent old inflation.

Guth's original model of inflation involves a first-order phase transition associated with spontaneous symmetry breaking (SSB) of a grand unified theory (GUT). SSB is affected when the scalar field ϕ acquires a vacuum expectation value, $\phi = \sigma$. At high temperatures ($T \geq T_c$, where T_c is the critical temperature for the phase transition) the finite temperature effective potential, $U_T(\phi)$, is minimized for $\phi = 0$, while at low temperatures ($T \leq T_c$) $U_T(\phi)$ achieves its minimum at $\phi = \sigma$, which signals SSB. For a first-order phase transition the symmetric state ($\phi = 0$) is still a local minimum of U_T for $T < T_c$, and is metastable for $T \leq T_c$ due to the potential barrier between $\phi = 0$ and $\phi = \sigma$. Because of this the Universe can exist in the metastable, symmetric state for $T \leq T_c$. While it does there is an enormous vacuum energy associated with the metastable (or false vacuum state), $U(\phi = 0) = 0(T_c^4)$ (where for $T \ll T_c$, $U_T \equiv U$ is temperature independent), and this vacuum energy drives an exponential expansion of the Universe (inflation). Because of the potential barrier between the false and true vacuum states, transition to the true vacuum must occur by the nucleation of bubbles of the true vacuum state which then expand at the speed of light¹⁰.

In the context of the Friedmann-Robertson-Walker cosmology Guth has calculated the probability that a given point in space remains in the symmetric phase and finds that it is $e^{-F(t)}$ where

$$F(t) = \int \lambda(t_1) R^3(t_1) V(t, t_1) dt_1$$

$$V(t, t_1) = \frac{4\pi}{3} \left[\int_{t_1}^t \frac{d\theta}{R(\theta)} \right]^3$$

$R(t)$ is the Friedmann-Robertson-Walker scale factor, and $\lambda(t)$ the nucleation rate,

taken to be constant once the phase transition has begun:

$$\lambda = \begin{cases} 0, & T > T_c \\ \lambda_0, & T \leq T_c \end{cases}$$

[Throughout we use units where $\hbar = k = c = 1$ and $G \equiv m_{\text{pl}}^{-2}$.] Inflation begins when $T \approx T_c$, at $t = t_{\text{GUT}} \approx 1/3\chi$, where $\chi^2 = 8\pi U(0)/3m_{\text{pl}}^2 = O(T_c^4/m_{\text{pl}}^2)$. During inflation, $R \sim e^{\chi t}$, and

$$F(t) = \frac{4\pi\lambda_0 t}{3\chi^3}$$

When $F(t)$ becomes of order unity, most of space is in the true vacuum phase, and the inflationary phase is over. A minimum of about 60 e-folds of inflation is required in order to solve the horizon and flatness problems². Since inflation ends at $t = t_*$ where $F(t_*) = 1$, this implies that for inflation to be successful the nucleation rate λ_0 must be $< 3\chi^4/4\pi N \approx \chi^4/80\pi$ where the number of e-folds of inflation, N , is taken to be ≥ 60 .

Consider the effect of initial anisotropy on inflation. Following Barrow and Turner, we assume that at early times, the energy density of the Universe is dominated by 'anisotropy energy density', Σ^2/R^6 , and that the analogue Friedmann equation for the mean scale factor $R(t)$ (valid for all Bianchi I models) is

$$\left(\frac{\dot{R}}{R}\right)^2 = \chi^2 + \frac{\Sigma^2}{R^6} + \frac{8\pi}{3} \frac{T^4}{m_{\text{pl}}^2}$$

where $\Sigma^2/R^6 \gg T^4/m_{\text{pl}}^2$, χ^2 represents the contribution of the vacuum energy, and the temperature $T \propto R^{-1}$. (This equation is only valid if the Universe is still in the symmetric state, for $t \leq t_*$.) At early times ($t \leq t_V = 1/3\chi$) anisotropy dominates and $R \sim t^{1/3}$, while at late times ($t \geq t_V = 1/3\chi$) vacuum energy dominates and $R \sim e^{\chi t}$. Ignoring the subdominant T^4 term, evolution of the mean scale factor $R(t)$ is given by

$$R^3 = \frac{\Sigma}{\chi} \sinh 3\chi t$$

Note that vacuum energy begins to dominate at $t \approx 1/3\chi$, independent of the level of anisotropy. This fact, or equivalently, that Σ^2/R^6 is independent of Σ , has been used⁶⁻⁹ to argue that anisotropy has no effect on the phase transition or inflationary process. This misses an essential point, the level of anisotropy does influence t_{GUT} , the time at which $T \approx T_c$ and bubble nucleation can commence. In the presence of anisotropy

$$t_{\text{GUT}} = \gamma^{-1/2}/3\chi = \gamma^{-1/2}t_V$$

where $\gamma \equiv (\Sigma^2/R^6/\chi^2)|_{T=T_c}$, that is, γ is the ratio of the anisotropy energy density to that in radiation when $T \approx T_c$. The effect of larger anisotropy then, is to push t_{GUT} to earlier times, so that bubble nucleation can commence sooner.

During the anisotropy-dominated phase, $R \sim t^{1/3}$ and $F(t) = 9\pi\lambda_0 t^4/80$, which implies that $t_* \approx (80/9\pi\lambda_0)^{1/4}$. If

$\lambda_0 > 720\chi^4/\pi$, then $t_* < t_V$, and the transition to the true vacuum state will be complete before the vacuum energy comes to dominate, thereby preventing inflation. But if this inequality is satisfied, the previous constraint on nucleation rate implies that sufficient inflation would not have occurred in the absence of anisotropy either. That is, anisotropy does not spoil inflation that would have otherwise been successful. It is also known that initial anisotropy does not adversely affect new inflation^{11,12}. As Barrow and Turner pointed out and others have since studied in more detail^{11,12}, more than 60 or so e-folds of inflation will damp any initial anisotropy, regardless of amplitude, to an undetectable level today. Old inflation then also solves the anisotropy problem.

The error made by Barrow and Turner occurred just below equations (5), (6) where they incorrectly interchanged t_{GUT} and t_V . In a radiation-dominated Friedmann Universe, both t_V and t_{GUT} are $O(m_{\text{pl}}/T_c^2)$, and t_V is independent of the level of anisotropy. However, as discussed above, in the presence of anisotropy $t_{\text{GUT}} \approx \gamma^{-1/2}t_V$. This led Barrow and Turner to conclude that by raising the initial level of anisotropy one could always make the Universe anisotropy-dominated at $t = t_*$, thereby prevent inflation. Let us be more specific. While the Universe is anisotropy-dominated the ratio of anisotropy energy density to vacuum energy density decreases as t^{-2} , so at $t = t_*$

$$[(\Sigma^2/R^6)/\chi^2]|_{t=t_*} = \gamma(t_{\text{GUT}}/t_*)^2$$

$$= (\pi\lambda_0/720)^{1/2}/\chi^2$$

which is independent of γ and only greater than unity if $\lambda_0 > 720\chi^4/\pi$, the same condition found previously. By mistakenly using t_V for t_{GUT} in this equation Barrow and Turner found that the ratio of anisotropy to vacuum energy density at $t = t_*$ to be γ times the correct expression, and concluded that sufficient initial anisotropy could guarantee that the Universe was anisotropy-dominated at $t = t_*$.

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1. Barrow, J. & Turner, M. *Nature* **292**, 35 (1981).
2. Guth, A. *Phys. Rev. D* **23**, 347 (1981).
3. Guth, A. & Weinberg, E. *Nucl. Phys. B* **212**, 321 (1983).
4. Linde, A. D. *Phys. Lett. B* **108**, 389 (1982).
5. Albrecht, A. & Steinhardt, P. J. *Phys. Rev. Lett.* **48**, 1220 (1982).
6. Grøn, Ø. *Phys. Rev. D* **32**, 2522 (1985).
7. Futamase, T. *Phys. Rev. D* **29**, 2783 (1984).
8. Demianski, M. *Nature* **307**, 140 (1984).
9. Moss, I. & Sahni, V. *Phys. Lett. B* **178B**, 159 (1986).
10. Coleman, S. & deLuccia, F. *Phys. Rev. D* **21**, 3305 (1980).
11. Turner, M. S. & Widrow, L. *Phys. Rev. Lett.* **57**, 2237 (1986).
12. Jensen, L. & Stein-Schabes, J. *Phys. Rev. D* **34**, 931 (1986).

Beating the big battalions

Publishers are not serving some of their authors or student customers as well as they might. Wallace S. Broecker describes his experience of desk-top publishing, and suggests how authors could make themselves a force to contend with.

IN MY subject, geochemistry, there is a dearth of textbooks designed for use in advanced undergraduate and first-level graduate courses; further, those textbooks we do have are often not written by the most appropriate people. Very often we are forced to teach entirely from articles published in journals, or from books with a large number of contributors, which is an inefficient and uninspiring means of presenting the subject. I suspect that the situation is much the same in many of the more specialized but rapidly growing fields of science and engineering.

There are several reasons for this state of affairs. One of them, I contend, is that the publishing industry has a number of practices which discourage creative authors. I list a few.

(1) More daring and inventive proposals are rejected because of the unwillingness of publishers to take risks. Their tendency is to follow the format for well-defined courses run at many universities; the result is books that are alike in content and design. This is being competitive but unimaginative.

(2) Once the manuscript is submitted, the publisher is inclined to view it as his property and dominates decisions about format, cover design, marketing and so on. The author's personality is removed by editing out whimsy and anything that seems unconventional.

(3) Publishers provide a professional service of copy-editing, production, marketing and distribution, but at the cost of supporting large overheads. They are also commercial organizations, interested above all in making a profit. The results are that books are expensive, especially those for the less well-populated advanced courses, and that authors rarely see a decent financial return for their efforts.

Is there an alternative? I have conducted two self-publishing experiments which demonstrate to me that there is. The first book, entitled *Tracers in the Sea*, is 700 pages long and is a treatise on dissolved chemical species in the sea. Since its publication in 1982 it has sold 3,000 copies. All the costs have been recovered; the account is now in the black. The second, *How to Build a Habitable Planet* (280pp.), is aimed at university students and non-scientists interested in the physical heritage of the Earth. Like the previous book it is distributed by Lamont-Doherty Geological Observatory, but because it was published in December 1986 as yet we have little indication of how well it will sell.

As never before, the combination of word-processor, computer layout and laser printing brings within every author's grasp the possibility of producing his or her own book. As those of us who use the 'author-ready copy' option for journal articles already know, this technology is in place. We have only to learn how to use it. The manuscript is typed and edited on a word-processor. The disk from the word-processor (including figure captions and tables) is transferred to a computer system, on which the page layouts are done and which also scans the line-drawings. A laser printer is then used to make camera-ready copy for the printer, who needs only to drop any photographs into position.

One might ask, "Isn't it painful to handle all the production details?" To me it was a pleasure after all the sweat I had put into the writing and revisions. The time required was surely less than 5 per cent of the total. Much of this went into proof-reading and other tasks with which all authors have to contend. Above all, the book remained mine.

Then, where's the rub? Why doesn't everybody do it? There are two big problems. One has to do with financial backing. In my case there was the matter of

Summary of production costs for *How to Build a Habitable Planet*

Typing and drafting (in house)	\$13,000
Photographic fees	1,000
Copy editing (freelance)	1,000
Computer layout (commercial)	3,000
Printing (5,500 copies)	12,000
Total	\$30,000

Financial outlook

<i>First printing (5,500 copies)</i>	
Production costs	— \$ 30,000
Distribution costs	— 25,000
Sales receipts	+ 117,000
'Profit'	\$ 62,000
<i>Second printing (5,500 copies)</i>	
Production costs	— \$ 12,000
Distribution costs	— 25,000
Sales receipts	+ 117,000
'Profit'	\$ 80,000

This calculation assumes that all the books will be sold, which of course they might not be. Herein lies the risk. However, most reputable textbooks should easily exceed the break-even sales point (for this book about 2,200 copies).

\$30,000 to be found (see the table on production costs). I borrowed the money from a combination of sources. As can be seen, the total production cost, including all the typing (I write by hand, in pencil, by rivers, in cafeterias . . .) and drafting, came to just under \$6 per book printed. To the extent that an author did his or her own drafting, typing and computer layout, the costs could be reduced significantly. We estimate the distribution costs to be about \$5 per book. At the discount price of \$18 for the softcover edition and \$30 for the hardcover, I must sell a mix of about 2,200 books before the break-even point is reached (see the table on financial outlook).

The other problem has to do with marketing and distribution. Herein lies the real pain. My first book was aimed at a sufficiently narrow audience and was unusual enough that a combination of help from friends, careful advertising and word of mouth did the job. The costs were repaid and I received a modest royalty. Further, at \$35 the book sold for less than one-half the price a publisher would have charged. This brought it into a range where students could purchase it out of interest and not only out of necessity. For my second book, the task will be more difficult. As there is no ready-made audience, I will have to work hard to create one.

Thus, although technology has removed the production problem, the problems associated with financing and distribution will force most authors to put themselves in the hands of a publisher. Part of the answer may lie in distribution co-operatives which cover a broad enough part of a given field to become viable. Participants would produce their own books (with advice from the co-op) and then use advertising and distribution services of the co-op. The other part of the answer may lie in grants from charitable foundations to provide the money required for typing, drafting, layout and printing.

I am convinced that one important step towards remedying the lack of quality textbooks in certain areas of science is to force the publishing industry to think again about the way it treats its authors and serves its student customers. This can be done through competition created by the alternative publishing route outlined above. The production technology is already accessible to most authors. What remains to be developed are mechanisms to permit aspiring authors to obtain the funds needed for the production of their books, and co-operative systems by which those books could be marketed and distributed. □

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Varieties of quantum lore

R. B. Jones

- Quantum Mechanics in Simple Matrix Form.** By Thomas F. Jordan. Wiley: 1986. Pp. 257. Pbk \$26.50, £25.70.
- Quantum Mechanics.** By Ta-You Wu. World Scientific: 1986. Pp. 417. £33.15, \$41.40.
- Non-Relativistic Quantum Mechanics.** By Anton Z. Capri. Benjamin/Cummings: 1986. Pp. 653. \$34.95, £36.95.
- Quantum Mechanics and the Particles of Nature: An Outline for Mathematicians.** By A. Sudbery. Cambridge University Press: 1986. Pp. 358. Hbk £40, \$60; pbk £15, \$25.
- Quantum Mechanics, 2nd Edn.** By A. I. M. Rae. Adam Hilger: 1986. Pp. 247. £9.95, \$22.

There are nine and sixty ways of constructing tribal lays, and every single one of them is right.

KIPLING was unaware of the tribe of quantum mechanics, but he unerringly described their prolixity in composing accounts of their arcane lore. Of the five well-produced and useful volumes reviewed here only two are straightforward textbooks, the other three having features or aims that are as surprising as they are interesting.

Quantum Mechanics in Simple Matrix Form is a charming book written for "gifted high-school students" and for "most people who read the *Scientific American*". In his first 16 chapters Jordan succeeds admirably in reaching this audience by using the simplest quantum system, the spinning electron, to illustrate the assumptions of quantum mechanics, to explore the ideas of Bohr and Einstein, and finally to explain how experimental tests of the ideas of John Bell have shown convincingly that quantum mechanics does not describe an objectively existing local reality in nature.

In the second half of the book the going gets rougher for the non-mathematical reader, as the use of matrix methods is enlarged to treat angular momentum, the hydrogen atom, and symmetry operations of rotation and translation. This, then, is not a conventional text. Quite apart from all those high-school students, however, it should be read by undergraduates doing technical courses in quantum mechanics and by professional quantum physicists who will find it a refreshing presentation of simple and profound ideas.

Ta-You Wu learned quantum mechanics with Goudsmit and attended the lectures of Heisenberg and Uhlenbeck; later, as a teacher in wartime China, he taught the subject to C.N. Yang and T.D. Lee. The latter luminaries have contributed forewords to their old teacher's fascinating textbook, in which he explains the subject by meticulously tracing its historical roots.

Familiar himself with all the details of the old quantum theory of pre-1925, Professor Wu leads the student through the astonishing successes of this period and then draws on an encyclopaedic knowledge of the literature of 1925–1928 to

teach the subject as it was discovered then. Separate chapters trace the miraculous and almost simultaneous discoveries of matrix mechanics and wave mechanics. All the basic models and techniques of a modern course are covered clearly, with particularly detailed presentation of the special functions used for the oscillator, angular momentum and the hydrogen atom. There are good accounts of perturbation theory, and a brief mention of scattering problems and concepts of the Green function.

In the last three chapters, Professor Wu draws on his specialist knowledge of atomic and molecular spectra to describe approximation methods for energy levels of many electron systems; included here is an extended treatment of how different angular momentum coupling schemes play a role across the periodic table. This book is probably too detailed, and in places too advanced, for a one-semester first undergraduate course. But it is highly recommended as supplementary reading for advanced undergraduates or for first-year graduate students.

Anton Capri's book is an extensive and modern treatment of nonrelativistic quantum mechanics suitable for a two-semester undergraduate or one-semester graduate course. Essentially all of the standard topics are covered, although with an unstated bias towards the physics of nuclei and elementary particles as compared with condensed matter physics.

The author makes a noteworthy attempt to introduce more mathematical detail than is usual in his discussion of the linear space structure of quantum mechanics. The ideas of Schwartz spaces, dual spaces and rigged Hilbert spaces, and the distinction between strong and weak convergence, are clearly introduced, and the treatment of standard problems and techniques is uniformly good. I was pleased by the discussion of the WKB method and of the Glauber approximation for scattering theory. Capri is aware of the quantum Hall effect, and includes a nice account of the effect of a magnetic field and gauge invariance upon electron motion. He concludes with an introduction to second quantization and a brief description of density matrix methods. This book should

be carefully considered for use in advanced undergraduate or early graduate courses.

Anthony Sudbery's *Quantum Mechanics and the Particles of Nature* is written for mathematicians who want to learn about elementary particle physics. After an introductory look at the particle jungle, with its shy inhabitants the quarks and gluons, there is a three-chapter exposition of ordinary nonrelativistic quantum mechanics; here the reader is expected to understand quickly how rigged Hilbert spaces, tensor product spaces and Lie groups play a role in the theory.

Then comes a glorious chapter on quantum metaphysics, in which no less than nine (not exhaustive) interpretations of the basic quantum postulates and of the idea of measurement are explored and compared. In spite of the fact that, as Richard Feynman says, "Nobody knows how it can be like that", these metaphysical problems continue to exercise physicists and philosophers and are well surveyed here. In the next chapter the particle jungle is mapped out in detail, from isospin to flavour spectroscopy, from colour to asymptotic freedom, from the Salam–Weinberg theory to grand unification and supersymmetry. Sudbery's description is generally sound, except that he seems to believe that the top quark has been definitively established whereas the experimental group who are alleged to have found it are much more modest in their claims. The last chapter introduces relativistic quantum gauge theory, including hidden symmetry and the Higgs mechanism.

This volume is not to be regarded as a textbook. Rather, it is a stimulating monograph on how we attempt to grasp intellectually the essence of the elementary building blocks of the Universe.

The second edition of Alastair Rae's *Quantum Mechanics* does not differ greatly from its predecessor, reviewed here in 1983. The changes include a more careful treatment of spin–orbit interactions and the Zeeman effect, elaboration of the discussion of degenerate perturbation theory and an improved account of the uncertainty principle. A set of detailed hints to help with the exercises has been incorporated as well.

Of the five books reviewed, this is the one best suited to a straightforward one-semester introductory undergraduate course. It contains much of the quantum material required for an Honours BSc physics course, and lacks only consideration of some topics of relevance to condensed matter physics such as the role of the electromagnetic vector potential in quantum mechanics and the effect of spatially periodic potentials. □

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Central concerns in physics

James Lowe

An Introduction to Nuclear Physics. By W.N. Cottingham and D.A. Greenwood. Cambridge University Press: 1986. Pp. 210. Hbk £25, \$44.50; pbk £8.95, \$14.95.

Nuclear Physics: Energy and Matter. By J.M. Pearson. Adam Hilger: 1986. Pp. 250. £20, \$44.

BOTH of these books are intended as introductory, undergraduate texts for courses in modern nuclear physics. Similar criteria have guided the choice of material in each case, but the end results are rather different.

In their book, Cottingham and Greenwood present an interesting selection of topics. The rationale behind the choice of them, as stated by the authors, is that as well as learning about the basic properties of the nucleus readers should acquire enough information to enable them to understand the problems of present-day nuclear technology and to make informed judgements on them. Thus, for example, compound-nucleus reactions, which are very relevant to nuclear power generation, are discussed in detail, while direct reactions receive much less attention. Similarly, the restriction of the account of excited states to those below 10 MeV precludes consideration of such topics as giant resonances, which have no practical application but have surely been important in the development of our understanding of the nucleus. By contrast, nuclear reactors are treated in some detail.

Nevertheless, this is in no way an applied physics textbook. The authors invariably make the basic physics clear, and particularly helpful is the brief description of the properties of elementary particles in the opening chapters, which puts the remainder of the book into proper context. The sections on nuclear astrophysics are also very welcome; the treatment both of stellar formation and of nucleosynthesis brings out clearly the basic physical principles of the subject. With the one reservation over the omission of certain topics, I can warmly recommend this volume. In the preface, the authors acknowledge their debt to their teacher, Sir Rudolf Peierls; his influence is, I think, often discernible in the book.

Pearson's text is also slanted towards applications, but here the emphasis is

much stronger. The author specifically avoids writing for the specialist student, and instead provides a background for what he calls the "large-scale manifestations" of nuclear physics, such as nuclear power generation and nuclear applications in medicine. It follows that his discussion is often more concerned with conveying facts than with emphasizing physical principles or the relation of a topic to the rest of physics. Occasionally, I feel that this is taken too far. For example, the account of gyromagnetic ratios of particles refers to the 'classical' value of unity. The experimental values for the proton, neutron and electron are then quoted without any real comment on their interpretation or on the significance of the closeness of the electron gyromagnetic ratio to 2.

Not all aspects are treated in this way, however: indeed, to give one example, the discussion of the independent particle model deals with the basic quantum

mechanics of the problem better than do many other books. Nor is the coverage of applied nuclear physics limited to man-made applications; a significant fraction of the book is devoted to nuclear astrophysics.

These two books, then, declare a common aim and are remarkably similar in the material they contain. If buyers have to choose between them, they must ask why they are studying nuclear physics. If it is primarily for the applications, in the nuclear industry or other areas, then Pearson's book will probably be the more useful. If it is to understand the basic principles and to appreciate the relation of nuclear physics to other branches of physics, while seeking some general education in the implications of the subject for everyday life, then the choice is likely to be Cottingham and Greenwood. □

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Brazilian plasmas

Alan Phelps

Fundamentals of Plasma Physics. By J. A. Bittencourt. Pergamon: 1986. Pp. 711. Hbk £55, \$83; pbk £22, \$32.

HITHERTO, textbooks on plasma physics have originated from authors in the United States, Europe, the Soviet Union or Japan, thus reflecting the main centres of research activity in the subject. The developing efforts in plasma physics elsewhere in the world will result in the appearance of texts such as this one from Brazil. The book is written for advanced undergraduate and first-year graduate students, and is also designed to be suitable for self-learning. The style and idiom have a North American flavour.

Until recently there was only a small number of plasma textbooks which were both appropriate for undergraduate use and which used SI units or their equivalent. Bittencourt's book is pitched at the right level for its intended readers, uses rationalized MKSA units and includes problems at the end of each chapter, and it should therefore be of wide appeal. The usefulness of the problems for both teaching and self-learning would have been enhanced if solutions had been included, though in partial compensation the problems are phrased in a helpful manner which often leads to the answer. More material is included than can be covered normally in one semester and the user therefore needs to be selective. This is not a disadvantage, but lecturers trying to give a balanced introductory course may find that the lack of description of experimental aspects and of the contemporary

methods of plasma diagnostics means that they have to draw upon additional sources of information.

The title is very appropriate, as the author does indeed address the fundamentals. Although the mathematics required is not above undergraduate level, the sheer number of equations embedded in the text implies that the book will be enjoyed more by theoretically inclined students than by experimentalists. Those intending to pursue research into space plasma physics should find the coverage is relevant to their needs.

There are some drawbacks. The exposition is clear, logical and academically satisfying, but at the price of conveying only in part the sense of excitement that characterizes plasma physics at present and that is so important in motivating the average student. No distinction is made between the cyclotron frequency and the traditionally defined Larmor frequency. Also, in grouping the main experimental efforts for achieving fusion — (1) open systems (2) closed systems (3) theta pinch devices (4) laser-pellet fusion — the author over-emphasizes the current importance to fusion of the theta pinch devices, while 'inertially-confined plasmas' would have been a better description for (4) and would then have included other drivers such as particle beams, as well as lasers. There are, too, a number of typographical errors, but no more than one would expect in a book of this length.

These are minor points. *Fundamentals of Plasma Physics* should be acquired by most science libraries, though I expect students will use it mainly to supplement their other textbooks on the subject.

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• Cambridge University Press has reissued in paperback Arthur Eddington's classic *Space, Time and Gravitation: An Outline of the General Relativity Theory*, which first appeared in 1920. The new edition includes a foreword by Hermann Bondi; price is £8.95, \$14.95.

Unearthly exercises

David W. Hughes

Astronomical Methods and Calculations.

By Agnes Acker and Carlos Jaschek. Wiley: 1986. Pp.343. Hbk \$34.95, £38.50; pbk \$26.35, £14.95.

SCIENCE is quantitative and astronomy is no exception. But astronomy has a special piquancy because the problem of measuring such fundamental parameters as mass, size, temperature and composition is all the more demanding when the objects of interest are so far away.

Take as an example the calculation of the distance between our Galaxy and its near neighbour Andromeda (Messier 31). In 1919 Lundmark used novae observations to obtain a value of 200 kpc ($1 \text{ kpc} \equiv 3 \times 10^6 \text{ km}$). Bright, non-variable stars indicated that it should only be 100 kpc. By 1922 Öpik had obtained a value of 450 kpc, and Lundmark's recalculation in 1925 gave 430 kpc. In the same year, using Cepheids, Hubble plumped for 275 kpc. Returning to the problem in 1948 Lundmark measured the distance to be 405 kpc and Baade in 1952 found a value of 500 kpc. The consensus today is that Andromeda is about 660 kpc away. This variation carries through to our estimations of energy output and has an effect on such basic cosmological quantities as the density of the Universe.

All astronomers need to understand the methods used to measure fundamental quantities and must be able to assess the reliability of the values obtained. The normal way of developing this proficiency is by confronting students with raw data and then sitting over them in the laboratory or observatory until they have worked through to an end result. Laboratory

supervisors have to rely on a range of texts to help them devise meaningful exercises, and *Astronomical Methods and Calculations* is another to add to their book list (assuming, that is, that they do not already have in their possession the original French edition, which was published by Masson in 1981).

Acker and Jaschek provide the bare bones of 112 exercises, together (thank goodness) with a brief résumé of the expected solutions. There are four sections. The first concentrates on traditional astronomy, stellar co-ordinates, the determination of time, refraction and stellar motion. The second deals with the Sun and the planets, measuring distances using radar and the transits of Venus, assessing masses, sizes, shapes, temperatures, albedos and solar parameters such as limb darkening and curves of growth. The authors then turn to the stars and our Galaxy, and consider such topics as rotation, temperature scales, luminosity functions, Cepheid distribution and interstellar extinction. The final section deals with galaxies, their shapes and spectra, spin, distances, masses, clustering and velocities.

The reader is presupposed to have a considerable knowledge of astronomy, physics and mathematics, and most of the exercises will need amplification before they can be put before the average university student. There is a fountain of good ideas here, but one in which references are few and cross-referencing is far too meagre. And accuracy remains a problem. Little help is given to enable readers to differentiate between systematic and experimental error, so they will still have difficulty, for example, in finding out how far away Andromeda really is. □

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Making short work of heat

Tony Guénault

Elements of Thermodynamics. By Martin C. Martin. Prentice-Hall: 1986. Pp.225. \$39.33, £42.10.

STUDENTS and teachers alike recognize the need for accessible textbooks, ones that cover a subject in not much more detail than the corresponding lecture course. Here we have a praiseworthy attempt to provide a text for a short course on thermodynamics for middle-year physics or engineering students. This is a worthwhile target area, although one in which there is competent opposition.

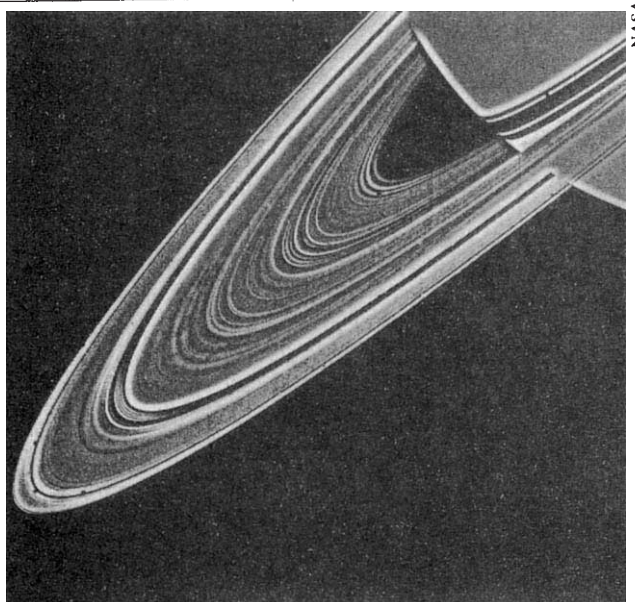
Martin's book has some strong points in its favour. The choice of topics is imaginative, and an introduction to the statistical approach is included. The level of the writing is well judged, although some readers might prefer fewer equations in places. And the production quality is very high, the work being well typeset and printed, making it a pleasure to pick up and read.

However, in spite of much to admire, this is not a book that inspires overall confidence. One problem is the basic approach to the subject. For example, it is surprising to discuss temperature before considering thermal equilibrium, and to see work introduced as something done by a fluid rather than against an externally applied (balancing) force. Throughout, more care and attention could have been given to the logical framework without affecting the level or length of the book. Thermodynamics should be an appealingly and remorselessly logical subject.

Given my own interests, and particularly because a picture of the fountain effect appears on the front cover, I turned quickly to the chapter on low-temperature physics. Here, one of the biggest disappointments is the illustrations. Several graphs look like artists' impressions of other artists' impressions, rather than depictions of the real thing. The phase diagram of helium-4 has a strange look to it, but that of the (λ ?) heat capacity is positively grotesque. And mention of the "more accurate" λ point of 2.189K makes one uneasy — a value around 2.17K has been accepted for some while now. Elsewhere there are other similarly unrealistic pictures (for instance of black-body radiation and of critical isotherms). These and other small unsatisfactory details rather spoil the impact of what is in some ways an attractive textbook. □

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In a whirl — the rings of Saturn, viewed from Voyager 2 in August 1981. The picture is reproduced from a colour original in the fifth edition of The Physical Universe, by Konrad B. Krauskopf and Arthur Beiser, published last year by McGraw-Hill.



Model exposition

Jean Palutikof

Contemporary Climatology. By Ann Henderson-Sellers and Peter J. Robinson. Longman/Wiley: 1986. Pp.439. Pbk £12.95, \$21.95.

IS THERE really room for another undergraduate textbook in climatology? I opened *Contemporary Climatology* with a somewhat world-weary sense of *déjà vu*, but was pleasantly surprised by the presentation, content and style. The book covers the full range of topics to be expected from a basic text, with useful sections on instrumentation and on the applications of climate information to economic (particularly agricultural) planning. The writing and the illustrations are of a high standard, and they combine well to ensure the reader's understanding of the subject matter. In addition, there is a helpful glossary of terms.

The most successful section is undoubtedly that on climate models. This is a rapidly advancing and increasingly important area of climate and climate-change studies; global-scale models are used to investigate problems as diverse as the nuclear winter (or autumn, if you prefer) and the Sahelian drought, while on a local scale models are used in applied climatology to look, for example, at water resources and crop-climate relationships. Yet adequate treatment of modelling theory and practice, particularly the long-term requirements of climatologists rather than the short-term forecasting needs of meteorologists, is rare in undergraduate texts. Here we are presented with about 40 pages of lucid explanation and illustration which should become the standard student reference on the topic. The other particularly enjoyable and educational aspect is the use of satellite images to illustrate points made in the text. Indeed, throughout the book frequent reference is made to the relevance of data gained from space to climatic research.

Overall, it is in the treatment of the most recent (particularly high-technology) developments in climatology that this book succeeds best. Conversely the weakest section is that on the most traditional branch of the subject — climatic classifications. The most annoying general feature is the failure to reference sources of information in either the text or figure captions; to follow up a particular reference the reader has to hunt through the Acknowledgements, a daunting task that few would pursue. But these are small criticisms of a book that seems destined to be widely used as a course text. □

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Ordered elements of sea and air

Malcolm Walker

The Atmosphere and Ocean: A Physical Introduction. By Neil Wells. Taylor & Francis: 1986. Pp.347. Hbk £29, \$52; pbk £15, \$27.

ACCORDING to the publisher's blurb, "*The Atmosphere and Ocean* is unique in bringing together the diverse concepts and ideas of meteorologists, atmospheric physicists and oceanographers into a single coherent account of our fluid environment, placing the emphasis on the physical ideas and mechanisms rather than on the mathematics". As co-author of a book with essentially the same theme (*The Ocean-Atmosphere System*, published by Longman in 1977), I was a trifle vexed by this claim; moreover, the designs on the front covers of the two books are similar, both adopting the waterspout as a motif to represent the coupling between ocean and atmosphere.

But these are minor complaints, not least because the styles of the books are very different. *The Ocean-Atmosphere System* largely takes the form of a review, whereas *The Atmosphere and Ocean* is an undergraduate textbook, concerned mainly with physical processes and demanding of its readers a greater competence in mathematics and physics. Indeed, without a subsidiary course in mathematics to introduce concepts not normally encountered at school, undergraduates

will surely struggle to follow some sections. The author no doubt assumed that such a course would be provided for students using his book formally.

On the whole, however, *The Atmosphere and Ocean* is an excellent supporting text for undergraduate courses in marine science. There is little to criticize: one or two symbols are not defined and a few explanations are perhaps over-concise. Apart from the occasional lapse of proof reading, that is all. The overall impression is one of a book over which a great deal of care has been taken, by both author and publisher.

For the most part, difficult concepts are explained clearly and the book is scientifically sound. Furthermore, the order of the contents has been carefully thought out. The ten chapters lead the reader logically from an account of the Solar System and solar radiation to a discussion of climatic variability and predictability, through studies of the composition and properties of the ocean and atmosphere, vertical temperature profiles, atmospheric and oceanic stability, water in the atmosphere, global budgets of heat, water and salt, ways of measuring winds and currents, the influence of Earth's rotation on fluid motion, the nature of waves and tides, and energy transfer in the ocean-atmosphere system.

In short, *The Atmosphere and Ocean* can be strongly recommended as a textbook for undergraduate courses in marine science. I expect it to be widely adopted as such. □

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Learning on a plate

J.R. Cann

Marine Geology: A Planet Earth Perspective. By Roger N. Anderson. Wiley: 1986. Pp.328. Pbk \$31.50, £21.80.

Plate Tectonics: How It Works. By Allan Cox and Robert Brian Hart. Blackwell Scientific: 1986. Pp.392. Pbk \$29.95, £19.75.

IT IS astonishingly difficult to find good textbooks for advanced undergraduate courses on plate tectonics. After all, the plate tectonic revolution has been more or less complete since September 1968, when the final touches of gilding were applied in a memorable issue of *Journal of Geophysical Research*. Yet the best sources for teaching are still the original papers themselves. "Come now, Joe", I hear all my plate tectonic friends out there saying, "that's exactly as it should be. Give the young people a chance to experience the excitement just as we felt it back in '63 or

'65!". But is this really the best way to teach? Remember the story that McKenzie and Morgan very nearly did not bother to publish their paper on evolution of triple junctions because they felt it was all too obvious — and that paper is still pretty nearly impenetrable, even for a bright student. And many of the classic papers have in one sense or another been superseded by later work, which is not tidily collected together anywhere. So yes, I do think there is a place for undergraduate texts on plate tectonics and related matters, and it is interesting to welcome two here, both written by practitioners of no mean talent themselves.

Roger Anderson's is the more conventional. It is a general account of marine geology, in some ways a condensed and more accessible version of Kennett's excellent *Marine Geology* (Prentice-Hall, 1982), but with considerably greater emphasis on solid-Earth aspects such as the geophysics of plate tectonics, and the geology of mid-ocean ridges and subduction zones. It is interesting that where Kennett's book is weaker, Anderson's is

stronger and vice versa. Thus in handling marine stratigraphy Anderson uses stock diagrams and makes subtle but definite errors in the handling of the biology and oceanography. On the other hand, his account of the subduction terrains of the western Pacific is entirely up to date, and illustrated with fine and striking graphics. It is unfortunate, though, that some of the diagrams in the book are reproductions in murky greys of colour originals, for which I fear the publisher must take responsibility. In addition to the more standard material of marine geology the book contains chapters on plate tectonics and continental geology, on the evolution of sedimentary basins and on mechanisms of plate tectonics. I shall be recommending this book to my students, and especially to those who are struggling with some of the more complex early papers.

Cox and Hart's book is very different. It is much more unconventional, taking a quantitative approach that is difficult to characterize in a word. Much of it deals with the geometry of plate tectonics, Eulerian poles, evolution of triple junctions (explaining McKenzie and Morgan very clearly), instantaneous rotations and finite rotations. But there are sections on earthquake focal mechanism solutions and on palaeomagnetism (not altogether surprisingly, considering the authors). Another way of seeing a unifying theme would be in terms of spherical geometry, which many of the topics in the book have in common. In some ways this theme is also the book's undoing, especially as finite rotations are gradually developed, and there are pages of detail that tend to obscure the flow of the argument. Another irritation is the folksy style of some of the linking prose — not that I'm against folksiness in itself, but when it is inaccurate (as in the fictionalization of a submersible dive) then I start to have doubts.

However this book is a most important addition to geological textbooks. It provides clear and systematic treatment of some of the most difficult and elusive aspects of plate tectonics, aspects which I suspect many people omit from their courses because of the problems of teaching them, but which are really fundamental to the subject. This is not a general text on plate tectonics, despite the title, but it is an excellent book that deserves to be widely used. □

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● *The Physics of the Earth's Core: An Introduction*, by Paul Melchior, is intended to provide a basis upon which astronomers, geodesists and others can build an understanding of the latest developments in the study of the Earth's interior. Publisher is Pergamon, price is hbk £26.75, \$39.95; pbk £13.35, \$19.95.

Resourceful science

Stephen E. Kesler *£33*

The Geology of Ore Deposits. By John M. Guilbert and Charles F. Park, Jr. W. H. Freeman: 1986. Pp. 985. Hbk \$47.95, £29.95; pbk £19.50.

Ore Deposit Geology. By Richard Edwards and Keith Atkinson. Chapman & Hall/Methuen: 1986. Pp. 466. Hbk £40, \$75; pbk £19.50, \$33.

Most geologists are descended from the early naturalists, whose interest in the Earth reflected purely intellectual curiosity. Not so the economic geologist, who traces his roots in part to the pragmatic prospector. Perhaps driven by a need to purge this non-scientific skeleton from our ancestral closet, modern economic geologists have emphasized the scientific aspects of the subject. Western students of economic geology rarely learn about the engineering and economic factors that make an ore deposit mineable, and they have little or no understanding of how to explore for new deposits. It is worthwhile contemplating whether this is partly responsible for the poor showing of many mineral companies during the lean years of the 1980s.

The two books under review reflect the schizophrenic heritage of economic geology. Guilbert and Park emphasize "the principles and data fundamental to understanding the genesis and localization of ore deposits", whereas Edwards and Atkinson attempt to "redress the balance between theory and practice" by focusing on "practical implications of the geological setting and genetic models of particular ore deposit types". Guilbert-Park, with over twice as many pages, would qualify as a reference book if the author-laden index could be improved. The difference in length between the two books reflects the amount of introductory material that precedes the ore-deposit descriptions, with only 17 pages in Edwards-Atkinson versus 306 pages in Guilbert-Park.

Both books wrestle with the perennial problem of classifying the wide diversity of mineral deposits and both adopt field-based schemes. Guilbert and Park organize their book around geological settings, whereas Edwards and Atkinson use the 'model' approach common in modern exploration thinking. The contents of both books reflect these classification schemes as well as the authors' backgrounds, with the Guilbert-Park system providing a more comprehensive, flexible coverage. Important deposit types, such as epithermal vein, sediment-hosted gold and magmatic iron deposits, are found only in Guilbert-Park, whereas sedimentary exhalative deposits are better covered in

Edwards-Atkinson. Deposits of recent interest, such as hot spring and detachment gold, are missing from both books. The classification of some deposits, such as volcanogenic massive sulphide under the "Magmatic Hydrothermal" class in Edwards-Atkinson, and Mississippi Valley-type lead-zinc and Athabasca-type uranium under "Epigenetic Deposits of Doubtful Igneous Origin" in Guilbert-Park, is confusing at best.

The main shortcoming of both books is the degree to which they integrate their approach to economic geology into the deposit descriptions. Edwards and Atkinson's persuasive call that more attention should be paid to the role of mining,



W. K. Bilodeau

Geological patterns — colloform banding in a sample of malachite from Zaire. The picture is reproduced from the book by Guilbert and Park, reviewed here.

metallurgical, economic and political factors in economic geology, is not matched by their coverage of these topics in the short introduction. The discussions of exploration-related aspects at the end of each chapter are also too short and generalized to be of use to students. Numerous topics ranging from middlings to induced polarization need to be described and related directly to the deposits if the approach is to succeed. Guilbert and Park do a better job of covering the geochemical and other research aspects of economic geology, with the exception of solubility controls on specific metals and minerals, but they do not use this information adequately in the descriptive sections of the book.

In spite of these shortcomings, both books contain a great deal of worthwhile material, are well presented, with clear illustrations and complete references, and would serve as satisfactory texts. Guilbert-Park would probably be the first choice of most geologists because of its much greater coverage, but if economic geologists are ever to stop being hired, and fired, by engineers, accountants and lawyers, they should also read Edwards-Atkinson. □

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World appearance

Edward Derbyshire

Earth's Changing Surface: An Introduction to Geomorphology. By M.J. Selby. Clarendon: 1986. Pp.607. Hbk £28, \$39.95; pbk £12.50, \$24.95.

OVER 20 years ago, Professor David Linton confessed that "geomorphology is, and perhaps always will be, one of the more difficult of the physical sciences". The root of this difficulty lies in the mixed conceptual base of the subject, which draws upon both historical analysis using a deductive framework, and functional studies which consider the behaviour of Earth materials and the processes acting upon and within the Earth's surface skin. The task of compiling a textbook to cover the whole field is thus one which demands deft handling of concepts and examples, so as to illuminate the explanations rather than give undue emphasis to the problems posed by the subject's diverse approaches, techniques and objectives.

On the face of it, much of *Earth's Changing Surface* is organized along traditional lines. There is an initial chapter on basic concepts, followed by four chapters on tectonics and volcanism as landform-shaping agencies, one on the properties of Earth materials, and nine using the familiar framework provided by the principal Earth-surface processes. The presentation of the first chapter is distinctive yet somewhat staccato: mention of the energy sources for landscape change, inheritance of landscape features, and time-scales precedes the definition of geomorphology which appears in a short account of the objectives and history of the subject a little further on.

The placing of a long section on climatic geomorphology after the historical account and before the brief treatments of equilibrium, geomorphic systems and applied geomorphology ("People as Geomorphic Agents") might bemuse the beginner for whom the book is intended. In fact, the progression is logical, but it could have been more plainly signposted. In contrast, the chapters on the main features of the Earth, global tectonics and structural landforms provide a clear introduction to global processes and their relationship to major landforms and structural features of the Earth, giving this subject an emphasis which has been called for in general geomorphology texts for some years. These chapters provide a wide range of basic information, from the classification of the igneous rocks to large- and small-scale structures, their resultant landforms and drainage patterns. Professor Selby effects the shift in scale down to weathering, hillslopes and the other process realms by inserting a chapter on the

behaviour, strength and resistance of rock, soil and water.

Many users of this book will judge it on the nine chapters devoted to process-landform relationships. I found them to be up to date, well illustrated and generally well balanced in content: the standard is appropriate for the beginning student with some basic science background. There is a minimum of mathematics, but, when used, it is fully supported by an explanation in the text.

Reaction to the last five chapters, which deliberately address broader issues, will vary. Their basis is largely climatic, yet 15 chapters separate them from the discussion of the principles of climatic geomorphology in Chapter 1. The chapter on Ice Ages can be read as a free-standing essay;

but to do justice to this closely written section, the student reading the succeeding chapter on planation and those on the landforms in the major climatic belts will require the guidance of a good teacher to ensure cross-reference to the earlier discussions of process relationships.

Professor Selby thus presents his subject matter in an interestingly different way. There is much to prod students into reading further, the last four chapters taking them beyond the introductory stage. This is an altogether impressive textbook, which will also serve well as a reference work for those requiring up-to-date views on landforms and landform change. □

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Burning issues

Ian Fells

Renewable Energy Resources. By John Twidell and Anthony D. Weir. E. & F.N. Spon/Methuen: 1986. Pp. 439. Hbk £30, \$65; pbk \$29.

Learning about Energy. By David J. Rose. Plenum: 1986. Pp. 506. \$59.50.

Renewable Energy Resources is designed to bridge the gap between descriptive reviews and specialized engineering treatises. It is intended for undergraduates in physical science and engineering and largely hits the mark, leaning towards the fundamentals of the subject with satisfying mathematical descriptions of the various phenomena: wind and wave power, solar heating and so on.

I suspect, however, that the innumerate renewable energy enthusiast, who feels instinctively that what is natural is good, will make no headway with this book, despite the sympathy of the authors. They point out in their preface that they enjoy the subject and hope their readers will also, and that practitioners must put on their rubber boots or sun hat and move outside. They might have added that a familiarity with vector analysis is also essential.

All of the principal renewable energy sources are analysed, and chapters on fluid mechanics and heat transfer are included to aid understanding. Practical details are largely absent, although a traditional charcoal kiln is described. There are quite searching sets of questions at the end of each chapter. Altogether, this is a useful textbook; my only criticism is that the word 'economics' does not appear in the index, and some analysis of costs would have put renewable energy resources into perspective alongside more traditional fossil fuels.

No such criticism can be levelled at

Learning about Energy, which "deals with energy as more than technology or economics or any other specific parts. It deals with energy as the fabric of civilization". The author's claim is substantiated by the arrangement of his material. The first two chapters are a general discussion about energy in a social context, in tune with his conviction that "energy is a means and not an end and stands for a series of activities engaged in by society in accordance with a variety of sometimes-conflicting goals". He chooses acid rain and the greenhouse effect as two examples of large burdens laid upon us by energy use. It follows that energy should be used effectively, and after explaining how this should be done the topics of oil, gas, coal and nuclear and solar energy are considered in some technical depth. Nuclear energy is picked out for particular examination; included here is an account of comparative risk assessment.

A puzzling omission, common to many books of this kind, is of any detailed treatment of the combustion process. Some 90 per cent of the world's energy is derived from burning some kind of hydrocarbon-based fuel, and the science and technology of combustion must be properly understood if it is to be carried out effectively. This book, for example, relegates combustion of pulverized coal in power stations to two paragraphs, although 15 pages are given to the structure and mining of coal. Combustion of oil and gas is not discussed at all. On the other hand, the economics of energy usage are well presented.

The treatment is entirely US-orientated, much of it in what the author quaintly describes as "Old English" units. The approach is mature and the book will be particularly appropriate for graduate courses. □

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In and out of the laboratory

Maurice Rigby £37

Experimental Physical Chemistry. By G. Peter Matthews. Clarendon: 1986. Pp. 495. Hbk £32.50, \$49.95; pbk £16, \$29.95.

A Textbook of Physical Chemistry. By A.S. Negi and S.C. Anand. Wiley Eastern: 1986. Pp. 972. Pbk £10.50.

Introduction to Physical Chemistry. By M.F.C. Ladd and W.H. Lee. Cambridge University Press: 1986. Pp. 347. Hbk £27.50, \$49.50; pbk £9.95, \$19.95.

THE changing face of physical chemistry is reflected in the 59 experiments selected for inclusion in Peter Matthews's book, the declared aim of which is to provide a practical course related to the material currently taught in degree courses. There is a good range of traditional experiments (including one or two venerable examples, such as the Dumas method of vapour density determination), but the results of recent technical innovation are well demonstrated in exercises such as laser Raman and Fourier transform infrared spectroscopy, microprocessor controlled pH titrations and molecular dynamics 'experiments' by computer simulation.

The book is in three parts, "Equilibrium", "Structure" and "Change", and is further divided into eight sections of grouped experiments. Each section has an introduction, putting the experiments in context, and each exercise is preceded by a summary of the appropriate theory and by a description of apparatus, experimental procedure and so on. Technical notes provide information for supervisors and technicians, including a guide to cost and complexity. Finally comes a list of selected references.

Among the experiments there are some interesting and unusual examples, such as

the measurement of the surface tension of liquid oxygen and a study of fluorescence quenching by electron transfer to metal ions. Equilibrium electrochemical measurements are perhaps favoured at the expense of dynamic studies (represented only by a polarography experiment) and the comparatively large number of exercises involving vacuum lines will tax the technical resources of some departments; indeed, it is likely that many departments will be unable to offer more than a limited selection of the experiments involving expensive or complex equipment. Nevertheless, there are numerous exercises which make more modest demands, and teachers planning a laboratory course, or looking for new experiments to add to an existing course, will find much of interest here.

In contrast to Matthews's book, there is little indication in the other two volumes reviewed here that much progress has occurred in physical chemistry during the past two or three decades. *A Textbook of Physical Chemistry* by Negi and Anand was written for degree students in Indian universities. The book follows a conventional sequence. It is characterized by the painstaking development of even elementary algebraic expressions, and offers little in the way of physical insights. There are many worked examples and large numbers of exercises at the ends of chapters, but the general standard of presentation is poor, with inconsistent notation and many typographical errors, by no means all of which appear in the six-page errata. The level reached is below that required for degree work in Britain or the United States, and the overall impression is of a rather old-fashioned text. For example, one must question the value of a nine-page exposition of the Bohr-Sommerfeld theory and four pages dealing with the parachor in a book that devotes less than thirty pages to the whole of molecular spectroscopy.

It seems unlikely that this book will

offer serious competition to established texts, such as Atkins, Barrow, Castellan, Moore and others. It certainly represents remarkable value in terms of pages per pound, though the quality of paper and typesetting reflect the price.

Ladd and Lee's *Introduction to Physical Chemistry* declares itself to be aimed at students in the first year of a chemistry degree course. It is developed from the same authors' *Modern Physical Chemistry* (Penguin, 1969) and parts of the text are identical with the earlier work. Many beginning students may well find the conventional single-volume comprehensive textbook daunting and would welcome an introductory text of this sort, but it is unlikely that the present book will meet their needs. The choice of material is unbalanced, with almost fifty pages devoted to the solid state but fewer than ten on the whole of spectroscopy, while the clarity of exposition leaves much to be desired. The account of atomic and molecular structure is extremely confused; the uncertainty principle is presented as arising from attempts to apply the Bohr theory to systems containing more than one electron, and the same theory is said to be unable to account for the bonding in methane and ethene. It will surprise most chemists to learn that this was an objective of the Bohr model!

The description of bonding theories is also inadequate, and matters do not improve greatly in the chapter on thermodynamics. Here the exposition is characterized by numerous imprecise (and occasionally incorrect) statements, and the whole section will increase rather than dispel the confusion often experienced by newcomers to the subject. In brief, one must regretfully advise students seeking a good introductory textbook of physical chemistry to look elsewhere. □

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Chemical confrontation — a snowblower pumps sodium carbonate onto a serious spill of nitric acid near Denver, April 1983. This is a popular picture; it appears both in the seventh edition of John R. Holm's Elements of General and Biological Chemistry (Wiley) and the second edition of General Chemistry, by Donald A. McQuarrie and Peter A. Rock (W. H. Freeman).



Bill Wunsch/Denver Post

Light for organic chemists

Andrew Gilbert

Introduction to Organic Photochemistry.

By John D. Coyle. Wiley: 1986. Pp.176. Hbk £26.25, \$31.95; pbk £8.35, \$13.55.

Organic Photochemistry, 2nd Edn. By J.M. Coxon and B. Halton. Cambridge University Press: 1987. Pp.243. £37.50, \$69.50.

IN THE late 1960s and early 1970s, the rapid development of organic photochemistry resulted in the appearance of a number of introductory textbooks on the subject. In the past ten years, however, reflecting the lack of commercial exploitation of the area, few organic photochemistry texts at any level or degree of specialization have been published. The new and widespread interest in synthetic applications should lead to a renaissance of the subject, and the time is thus ripe for the writing of new texts which take account of recently accumulated knowledge of photoinduced organic reactions.

The books under review are both intended as introductions to organic photochemistry and they achieve this purpose most satisfactorily. Both are well-written and up-to-date with clear presentation of well-chosen material. But although the authors cover much the same ground, and at an appropriate level for undergraduates, they have organized their subject matter rather differently.

Most organic textbooks present the material according to functional group and this is the approach successfully used by Coyle. Thus, following the first chapter which outlines the general features of photoreactions, there are chapters dealing with the photochemistry of alkenes and related compounds, carbonyl compounds, and arenes, while the final chapter has sections on the photoreactions of all other chromophores. Coxon and Halton's book, the successor to a volume published in 1974, has a similar first chapter to that of Coyle, but the photoreactions are presented quite differently. There are two chapters on intramolecular reactions of the alkene bond and of the carbonyl group, and then a chapter on intermolecular cyclo-addition reactions of alkenes, carbonyl compounds and arenes. The final chapter deals with oxidation, reduction, substitution and elimination processes. Inevitably the last chapter in both books is somewhat of a mixed bag, but personally I prefer presentation by chromophore type if only because the enormous amount of carbonyl photochemistry is in one chapter rather than spread through three. Having noted this, it must be said that within the confines of their

chosen approach Coxon and Halton do discuss and illustrate the material in a readily understandable manner.

Coyle's book is intended for a general readership having a knowledge of basic organic chemistry, and the author considers that the theoretical bases of pericyclic reactions and Woodward-Hoffmann aspects are more appropriately dealt with in a general text. In contrast, Coxon and Halton intermingle explanations of these latter topics with the organic photochemistry; while this will appeal to undergraduate and postgraduate students, it does tend to break up the readability of the text for those who simply wish to gain a general appreciation of the photoreactions of organic compounds.

The two books also differ in the way that help is given to the reader to pursue the subject in more detail, although both approaches are satisfactory. Coxon and

Halton give specific references to review articles and the primary literature throughout their text, whereas Coyle provides a list of further reading at the end of each chapter and adds a useful note to each item outlining the subject matter covered. Neither book includes questions and problems, which is surprising but not a drawback; in my view such sections are rarely used by students.

Newcomers to organic photochemistry will benefit greatly from reading either of these texts. Both provide a sound introduction to the subject, and prospective buyers will have to make their own choice according to cost and their personal preferences for the detailed content and organization of the material. □

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Diverse problems

Selby A.R. Knox

An Introduction to Organometallic Chemistry.

By A.W. Parkins and R.C. Poller. Macmillan, London/Oxford University Press, New York: 1986. Pp. 252. Hbk £22.95; pbk £9.95, \$18.95.

THE organic chemistry of metals is a huge field, extending from lithium at one end of the Periodic Table, through the p- and d-block elements and the lanthanides, to the actinides at the other. One of the great attractions of the subject is the consequent diversity of structure, bonding, synthetic methods, reactivity and applications that it presents. Yet this very diversity makes any attempt to cover the whole field in a relatively short book, as here, a difficult one. In also aiming to provide both an undergraduate textbook, and specialist information for research workers, the authors made their task even harder. The result is disappointing.

Because the fundamentals are so different, the organometallic chemistries of main group and transition elements are normally taught separately, but as closely related areas. The authors have chosen an integrated approach but this often demands an artificial organization of the material under generalized, unhelpful headings in a forced attempt at harmony. Thus, for example, we find quadruply bonded divanadium compounds bracketed with antimony and bismuth alkyls, lithiation of arenes and *ortho*-metallation by transition elements taken together, and the synthesis of alkylidene complexes under "Reaction of Organometallic Reagents with Unsaturated Compounds".

But it is the superficial treatment of the basic aspects that is most disturbing. We

learn only that the 18-electron rule is empirical (this is even "stressed") and are given no understanding of its basis or the reasons for the existence of 16-electron compounds, so important in the application of organometallics to catalysis. Within the 250 pages, only three are devoted to the vital processes of oxidative-addition, reductive-elimination and migratory insertion, while β -elimination is hidden in sections entitled "Reaction of Organometallic Reagents with Metal Halides" and "Adducts of Organometallic Compounds"; the account of stereochemical non-rigidity warrants four pages. One even searches in vain for any thorough discussion of the way in which the reactivity of an alkene can be modified through coordination to a transition metal. The classic story of cyclobutadiene stabilization by complexation merits only a brief mention, and the wider issue of such stabilization of unstable organic molecules and the reasons for it are not addressed.

Irritatingly, the term 'insertion' is used throughout the book for reactions which are now known to proceed in reality by migration, and although this is explained on one occasion the many diagrams which involve this pervasive process are wrong mechanistically in depicting an insertion path. This confusion is most apparent in the chapter "Catalytic Applications of Organometallic Compounds", where hydroformylation is also described in an often inaccurate fashion.

In attempting too much, and thereby being driven to brevity and superficiality, the authors have produced neither a particularly good introduction to the field nor one which serves as a valuable source of information. As it stands, the book will not appeal to a wide audience. □

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An atmosphere of accessibility

Robert A. Duce

Air: Composition and Chemistry. By Peter Brimblecombe. Cambridge University Press: 1986. Pp. 224. Hbk £25, \$39.50; pbk £8.95, \$14.95.

Air: Composition and Chemistry is part of the Cambridge Environmental Chemistry Series, the goal of which is to provide a central core of texts for undergraduates and first-year graduate students in environmental chemistry. The early chapters briefly summarize physical properties of the atmosphere and discuss the chemistry of the lower atmosphere, including sources, sinks and residence times for atmospheric species, atmospheric photochemistry, aerosol chemistry and cloud/precipitation chemistry. Later chapters cover sources and effects of air pollution, and the chemistry of the upper atmosphere and of planetary atmospheres. While used sparingly, references are up-to-date, with many published in 1983 or later, and several review articles or books are listed in each chapter for further reading. No problem sets are given.

A book of 200 pages could not hope to

cover such a broad range of topics in great depth. Therefore the author generally follows a non-rigorous, descriptive approach, which suggests that the students using the text are not necessarily expected to have a strong scientific background. In most sections this approach works well, and the author has generally succeeded in making the topics relevant and interesting to non-specialists in atmospheric chemistry. However, at times simple explanations are combined with rather complicated mathematical equations. Often these equations are not used nor their derivations presented, for example the complex equations for calculating plume dispersal and Rayleigh and Mie scattering. Such terms as vibrational and rotational changes, spin conservation, terpenes and Gaia hypothesis are used with no explanation, which may also be confusing to non-specialists.

There are several notable omissions. There is little mention of aerosol particle residence times, and the discussion of indoor pollution does not address current

concerns about radon and asbestos. After stating that the atmosphere is in steady-state chemically, it is pointed out that the concentrations of several gases are increasing globally, including carbon dioxide, methane and nitrous oxide; however, the potential impact of the increase of these and other trace gases on future climate and on the oxidizing capacity and photochemistry of the global atmosphere is only hinted at.

The book is refreshingly free of typographical errors and will be a very useful supplemental text for atmospheric chemistry sections of undergraduate environmental chemistry courses. It gives a broad and generally well-integrated picture of our current understanding of atmospheric geochemistry and air pollution chemistry. But I would not recommend it for an in-depth undergraduate or graduate course in atmospheric chemistry. □

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Chain effects

Paul Calvert

Introduction to Physical Polymer Science. By L.H. Sperling. Wiley: 1986. Pp. 439. \$39.50, £38.05.

A TECHNOLOGICAL proverb says that whenever you open a can of worms the only way to get them all back in is to use a larger can. It is the same with textbooks, where the need to update some topics raises the difficult question of what to expand and what to drop.

The author of this book, L.H. Sperling, is at Lehigh University where physical polymer science is a final-year undergraduate course offered along with polymer chemistry and practical courses. If we take our original can of worms as being Billmeyer's *Textbook of Polymer Science* (Wiley; 3rd Edn, 1984), then Sperling has dropped the polymer chemistry, as his title implies, and also the survey of commercial plastics and processing methods. Mechanical properties are treated in the final chapter but in a rather sketchy way. In return for the omissions we get material on neutron scattering, chain packing and folding, and reptation, as well as detailed accounts of crystallization kinetics and glass-transition theories.

The approach is often original, but the development of the subject matter is clear and progressive. After an introductory survey, chain configuration is discussed with reference to NMR and IR spectroscopies. The chapter on molecular weights covers osmometry, scattering methods,

viscosity and gel permeation chromatography, and also neutron scattering. Solution behaviour is covered from the standpoint of phase separation and polymer blends.

The amorphous and crystalline states are treated on the basis of neutron scattering data, and of packing models, and there is a thorough description of crystallization kinetics with nucleation theory and chain folding. Reptation, too, makes an appearance here.

The glass transition is dealt with in some detail but the treatment is fairly traditional; likewise, the chapter on rubber elasticity. The discussion of viscoelasticity and flow is rather disappointing, in that it concentrates on springs and dashpots where I would have liked to see more of the Rouse model and reptation.

In my view the distinctive characteristics of polymers are chain configuration, the random coil and entanglements. Sperling does a good job on the first, treats the second adequately and omits the third. Glass transitions and crystallization are not peculiarly polymeric but must be dealt with in polymer physics because most students do not see them elsewhere. In such a course it is easy to try to cover so much that nothing is done in depth, and for this reason I favour the space given to theories of crystallization and the glass transition. □

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• Recently published by Wiley Eastern is *Polymer Science*, by V. R. Gowariker *et al.*, a 500-page textbook which covers all aspects of the subject. Price is £38.50, \$39.95.

THE MANAGEMENT OF AIDS PATIENTS

Edited by DAVID MILLER, JONATHAN WEBER and JOHN GREEN

The Management of AIDS Patients is the first comprehensive guide to the practical clinical management of patients with AIDS or HTLV III infections. The book avoids the sensational aspects of the disease, offering solid advice and information for all people involved in patient care.

The editors and many of the contributors come from St Mary's Hospital, London one of the foremost centres in Britain for the treatment of AIDS patients. The knowledge and experience of these experts has been combined to provide a much-needed book for doctors, nurses, dentists and indeed for health-care professionals everywhere.

1986

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M MACMILLAN PRESS

What's the use of understanding?

K.A. McLauchlan

NMR in Chemistry: A Multinuclear Introduction. By William Kemp. Macmillan, London: 1986. Pp.240. Hbk £25; pbk £11.95.

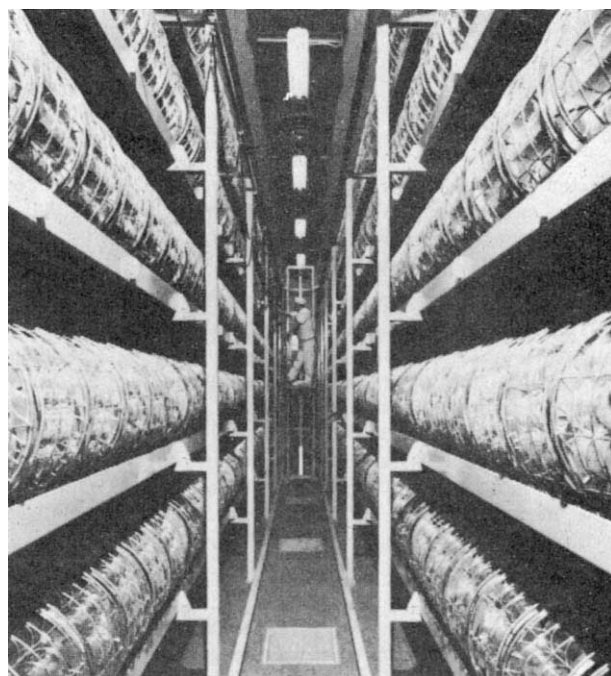
THIS is another attempt to introduce NMR and its chemical applications to undergraduates, but on a wide front. Basic chapters of the book are concerned with fundamentals, spectrometer design, 'advanced' theory, multi-pulse and computational methods and dynamic aspects, whilst individual chapters are devoted to spectra from ^1H , ^{13}C , ^{19}F , ^{31}P , ^{14}N and ^{15}N , ^{17}O and 'other' nuclei. A particularly bizarre chapter covers background physics, and provides a 'biography of NMR' which includes those unlikely magnetic resonance scientists Arrhenius and Van't Hoff but misses out some of the major figures. Tables of reference data are provided on ^1H and ^{13}C spectra (and the Greek alphabet!).

It is a self-satisfied book, the closest approach I have yet seen to a coffee-table textbook. Content has yielded to presentation and I should resent paying good money for several half-empty pages, pictures of archaic equipment and gyroscopes, and photographs of the periodic table (if it existed) in 1808. In a postscript of great banality entitled "Nocturnal Processes" (on which title I restrain myself from commenting), the author is quite open about his philosophy. Paraphrasing its content, the idea is that one does not have to understand a subject to use it — not an attitude I immediately sympathize with, although it might occasionally be justified.

However, even if students are to use NMR without understanding it, what they are told about it must be correct and the material must be presented by someone who does understand it, in depth. The author displays too shallow a knowledge to guide others, as is shown by many infelicities. For example, spin-lattice relaxation is said to be caused by electric dipole interactions; remarks made about quadrupolar broadening, contact and pseudo-contact interactions and so on are largely incomprehensible; and many of the basic ideas of the origins of the NMR parameters are stated in a needlessly confusing manner. This is rather a pity, for at times the author's approach works quite well. As it is, the book cannot be counted a success. □

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Roundly efficient — one of four incubators employing the 'rolling bottle' system for the production of monolayer cells at the Istituto Zooprofilattico Sperimentale, Brescia, Italy. The picture is reproduced from Basic Biotechnology, reviewed below.



Bugs and brass

Stephen Oliver

Basic Biotechnology. Edited by John Bu'Lock and Bjorn Kristiansen. Academic: 1987. Pp.561. Hbk £57, \$85.50; pbk £20, \$29.95.

BIOTECHNOLOGY is a diverse subject with vague boundaries. It is not just recombinant DNA technology, but includes chemical and control engineering, microbiology, biochemistry, chemistry, instrumentation science and genetics. The assembly of a text which covers the basics of so vast an area presents a formidable problem. Many previous attempts have resulted in huge, multi-volume compendia which no student and few libraries can afford. This book attempts to take a hard-nosed (or, as the editors would have it, "brass-nosed") approach and strips the subject down to its essentials.

This is achieved by dividing the book into two parts — "Fundamentals and Principles" and "Practical Applications". In Part I, authors from the individual disciplines each give accounts of the basics which relate to biotechnology. These provide an excellent grounding for students with a background in the biological sciences; kinetics, thermodynamics and mathematical modelling are all well explained. They are not so appropriate for engineering students, however, because the principal actors in the drama appear to have left the stage. We learn little of the microorganisms themselves; there is nothing on cell structure, growth habits or life cycles. The engineering student is presented with a masterly account of microbial metabol-

ism and will learn, for example, of all the mitochondrial pathways without actually being told what a mitochondrion is. Similarly, topics such as gene regulation and the cell cycle are given scant coverage.

In Part II, the intention is to demonstrate how the various disciplines are integrated to produce efficient industrial processes. In some chapters, for instance that on biotransformations, this works well. In others, the authors have tried to be too comprehensive, and consequently have produced pieces which tend to be catalogues. The last three chapters, on genetic engineering, and animal and plant cell culture, are rather more forward looking than the rest. But even here there is an emphasis on what has been firmly established and successfully implemented. In the contribution on genetic engineering, for instance, there is no discussion of site-directed mutagenesis.

Each chapter provides a reading list rather than a full bibliography. The references given are to reviews and books published in the 1970s or very early 1980s, which must themselves have been dated when they appeared, and pointers to the current literature would have been welcome.

This text, then, is indeed basic and brass-nosed. It is also authoritative, and will provide students from the biological sciences with a firm foundation in biotechnology. I will direct them towards it but, at the same time, will point out that if they are to contribute to the future of the area they would do well to look up into the blue skies, and check that there is enough brass around to back them. □

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Developing concepts of biology

David R. Garrod

Developmental Biology. By William F. Loomis. Macmillan, New York/Collier Macmillan, London: 1986. Pp.405. \$33, £22.

"O THIS learning! What a thing it is!" And how may it best be accomplished? In this textbook for students of developmental biology, Loomis adopts an interesting approach that may be described as a tall and thin. In 400 pages, he takes the reader from the most elementary rudiments to much more advanced and up-to-date aspects of the subject, going into depth only with a few specific samples.

After a brief historical introduction, the text is divided into three sections: "Mechanisms", "Processes" and "Chosen Systems". The first deals with genetic and physiological aspects of cellular differentiation, the second is really descriptive embryology, and the third considers in more detail particular topics (such as limbs and pattern) or well-studied organisms (such as *Dictyostelium*, *Drosophila* and *Caenorhabditis*). Together with the "specific examples" given at the ends of the earlier chapters, the later chapters give the book its strength: in general they are good and readable. The other

aspects, particularly the descriptive embryology, have been frequently covered elsewhere, and often rather better. Among the specific examples, there is a distinct bias towards the control of gene activity and differentiation, which are well done, while other developmental topics, such as the control of morphogenetic movements, cell motility and adhesiveness, have much less emphasis.

The text is clearly and straightforwardly written, and is easy to follow. The illustrations, however, are for the most part the same old ones that could now justly be described as hackneyed; they may be appropriate for a textbook, but they do not fill one with enthusiasm. Learning aids are provided in the form of tables giving guides to the subjects covered (really a supplement to the index), helpful study questions and reading guides, and brief summaries in each chapter which are rather less useful.

Developmental Biology is certainly worth considering for teaching purposes, especially if one is looking for a book that will take the student through the various levels of study. Loomis has made a brave attempt at a difficult job, but perhaps one ought to conclude that, as with virtue, "There is no road and ready way to" learning and teaching developmental biology. □

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Look to tomorrow

J. Robert S. Whittle

Gene Activity in Early Development, 3rd Edn. By Eric H. Davidson. Academic: 1986. Pp.670. \$49.50, £41.50.

Genetic Analysis of Animal Development. By Adam S. Wilkins. Wiley: 1986. Pp.546. £66.95, \$69.95.

Foundations of Developmental Genetics. By D.J. Pritchard. Taylor & Francis: 1986. Pp.372. Hbk £33, \$63; pbk £14.50, \$33.

A GREAT deal hangs on the outcome of the genetic analysis of developmental systems. Evolutionists expect to learn about the type and magnitude of functional changes in genome structure that accompanied diversification of animal forms; neurobiologists hope for coherent ideas about the generation of nervous systems to parallel the sophistication with which they can examine functional connectivity in neuronal circuitry; protein and membrane biochemists want to be sure that they are facing up to the most relevant facets of behaviour of molecular assemblies in cells; and theoretical biologists will

be interested to know whether there will be enough information to construct an algorithm or a generative model, and whether it is still worth importing complex system analysis. As well as offering a powerful expansionist technology, molecular biology has brought to genetics the attention of these neighbouring disciplines because the promise of a general understanding of developmental processes is in the air.

The promise of resolution may prove to be false, but how will these three books encourage a student to understand the excitement and anticipate progress in this area? They are not really competitors. The declared and achieved scope of each of them is different, their styles are distinct and they may epitomize different philosophies.

Davidson is a fundamentally recast and expanded third edition of his 1968 book (then of only 375 pages). I agree with his own description that it contains "more molecular and genetic evidence that is of biological import than did its predecessor". It is a meticulously prepared heavyweight review, clearly written but with the density of citation and numerical detail that are the stuff of reference works. Having seen how well it treats the area

familiar to me, I regard it as an invaluable insurance against having to read more than a fraction of the 1,800 or so references cited. It would make a tough but rewarding Bible for a one- or two-semester series of graduate discussion seminars. It is no textbook but comparison with the other two volumes illuminates all three.

Taking only the "genetically tractable systems" *Drosophila*, *Caenorhabditis* and *Mus*, in his book Wilkins gives a masterly presentation of the internal logic and working methods that geneticists use to 'dissect' developmental processes. His style encourages participation in the deliberation within each case study, so that the reader appreciates the experimental strategies and judgements involved as well as the specific conclusions. Wilkins is definitely in charge of the discussion, dealing sensibly, as Davidson does discreetly, with the recurrent metaphor of the genetic programme and choosing an operational definition of determination to avoid having to satisfy legion other armchair definitions. If we don't stop and think occasionally, we become kleptomaniac about data, but as a geneticist Wilkins also knows there is a constant threat that discussion may descend into metaphysics. He avoids it. The detail in his expositions might well be from the research seminar or the conference coffee break, and provides a logical and explicit continuity that is frequently absent from research papers.

Pritchard has broader horizons. First, he draws material from more experimental systems, in particular from vertebrates (his own research is on transdifferentiation in the vertebrate eye). He raises global issues of adaptation and evolution in developing systems, discusses genetic assimilation and atavism, and repeatedly encourages the reader to wider perspectives. He then offers an interesting synthesis in Chapter 14, under the title "Principles of the Adaptive-Inductive-Genetic Model", that attempts to encompass many of these classic case studies. The order of topics is unusual, and deliberately so, moving from 'classical' embryological phenomena to differentiation and then on to several chapters on the machinery of gene expression (RNA, chromosomal proteins, transcription) and then to growth and morphogenesis.

In a course on development in 1963, part of my own genetics degree, I recall a bizarre *mélange* of phenomena (serotypes in *Paramecium*, homoeotic mutations, X-chromosome inactivation, genetic assimilation and position-effect variegation) which were supposed to come in handy one day or, as I judge now, become albatrosses for any grand synthesis. I sense that Pritchard might have preferred to enlarge upon some of his material (for example, the Leader cell hypothesis for transdifferentiation) but felt that convention re-

quired him to take space for groundwork. This, unfortunately, has constrained elaboration of his epigenetic standpoint (he was a student of Waddington and the book reflects Waddington's philosophy). On the other hand, Pritchard provides an introduction to developmental phenomena way beyond the territory of geneticists. It was courageous to introduce recapitulation, perhaps as a hostage to fortune, because we don't know whether this long-standing idea of ontogeny recapitulating phylogeny reflects a common developmental 'syntax' operating in diverse embryos and whether it is worth pursuing.

To be useful today, these texts must be forward-looking. But it is difficult to guess the shape of any grand synthesis until we have assimilated the impact of the currently fashionable tools in investigating gene expression in development. *In situ* probes for transcripts and protein gene products allow us access to the molecular anatomy of embryos; 'foreign' promotion of genes and of anti-sense constructs asks where the *absence* of gene expression is significant; iterative hunts for DNA sequences related to existing cloned genes are short-circuiting mutational dissection, while computer searches of virtual amino acid sequences from clones and sequenced DNA are identifying protein functions (EGF, homoeodomains, serine proteases) in genes previously known only by their mutations; and fission yeast seems the place to examine certain fundamental cell-division functions of the human genome.

The choice is not resolved. Pritchard offers a provocative perspective on developmental systems in the broadest terms but will require additional teaching commentary. Wilkins has the fluency and depth in the current technology and logic used in the three organisms that are going to remain field-leaders for geneticists. And for reference the sections in Davidson on cytoplasmic localization and lampbrush chromosomes are unmatched. Finally, may I record my hope for a more imaginative pricing of a paperback edition of Wilkins. □

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Spring books

The next book review supplement to appear in *Nature* is Spring books, which comes out in the issue of 30 April.

Among the books to be reviewed are *Time's Arrow, Time's Cycle: Myth and Metaphor in the Discovery of Geological Time* by Stephen J. Gould; *Philosophy and the Brain* by J. Z. Young; *The Communist Party and Soviet Science* by Stephen Fortescue; *Extinction* by Steven M. Stanley; *Never Satisfied: A Cultural History of Diets, Fantasies and Fat* by Hillel Schwartz; and the autobiography and biography, respectively, of Luis Alvarez and Isidor Rabi.

Genetic renewal

D.J. Cove & J.A. Stewart

An Introduction to Genetic Analysis, 3rd Edn. By David T. Suzuki, Anthony J.F. Griffiths, Jeffrey Miller and Richard C. Lewontin. *W.H. Freeman: 1986. Pp.612. Hbk \$35.95, £35.95; pbk £22.50.*

Concepts of Genetics, 2nd Edn. By William S. Klug and Michael R. Cummings. *Scott, Foresman & Co, Glenview, Illinois/Eurospan, London: 1986. Pp.699. \$28.76, £17.50.*

THE new editions of these two texts are not radically different from their predecessors. Both adopt a strictly classical approach to the teaching of genetics, dealing with eukaryote transmission genetics and cell division before molecular genetics. In both, mendelism is introduced with Mendel and population genetics is relegated to its familiar terminal position. Klug and Cummings place quantitative genetics where it logically belongs, following the analysis of the inheritance of discrete characters, but their treatment of this subject and particularly of heritability is patchy and not as good as that of Suzuki *et al.*

In view of the central relationship of evolution to genetics, both sets of authors adopt a surprisingly low key approach to the subject. Suzuki *et al.* are particularly coy; only two page references appear in

their index, and one of these is to the summary of a new chapter on transposable genetic elements. The summary begins "Nature has devised many different ways of changing the genetic architecture of organisms" and goes on to mention "selfish DNA", but this topic is not alluded to elsewhere in the chapter or in the book. Authors in the United States may feel it necessary to adopt this approach to placate the Creationist lobby, but if this is so it is to be regretted and diminishes the usefulness of their books.

For those who want a traditional approach to genetics, Suzuki *et al.*, with its excellent diagrams and illustrations, remains a strong contender for the textbook of choice. The index, however, is poor. Neither of these books is as useful as a reference work as M.W. Strickberger's *Genetics*, published by Macmillan in the United States and Collier Macmillan in Britain, which went into a third edition in 1985. Strickberger may be less suitable for self-instruction, but on almost every test it emerges as better for reference. Topics as diverse as punctuated equilibria and ribosome binding sites appear only in Strickberger, and most subjects are easily accessible by way of the index. It is principally for this reason that Strickberger will remain the general genetics textbook that we recommend to our undergraduates. □

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Access to theory

Joe Felsenstein

Population Genetics. By Freddy B. Christiansen and Marcus W. Feldman. *Blackwell Scientific: 1986. Pp.196. Pbk £14.80, \$19.95.*

Basic Concepts in Population, Quantitative, and Evolutionary Genetics. By James F. Crow. *W.H. Freeman: 1986. Pp.273. Hbk \$28.95, £28.95; pbk \$15.95, £15.95.*

POPULATION genetics has arguably the most complex body of theory in biology. For that reason the subject has never been easy to explain to biologists, who are often uncomfortable with mathematics and are usually reluctant to concede any role for theory in their science. The rise of molecular biology, with its paradigm of on-off switches, has directed attention away from quantization, as has the recent scepticism directed at neo-darwinian theory. Yet the molecular data themselves preserve information connecting the genes of different populations and different species. As the tide of molecular sequences rises, and as computers lead increasingly to the quantization of tra-

ditional morphological studies, the need for biologists to apply microevolutionary theory becomes greater.

Fortunately, successive textbooks have made the theory steadily more accessible. The two books reviewed here continue this trend. Both are available as paperbacks and are aimed largely at an undergraduate market. Christiansen and Feldman start with an overture, taking one-third of their book, on the description of variation in natural populations. The many distributions shown give an impression of concreteness which I found refreshing. But inevitably less room is left for the exposition of the detailed structure of the theory of changes in genetic composition of populations. Among the parts of the theory that are done less than justice is the decomposition of genetic variance of quantitative characters.

With smaller type and more pages, Crow's book is nearly twice as long, and proportionally more of it is spent presenting the theory (for example, the treatment of selection occupies 39 pages against 18 in Christiansen and Feldman). Although the writing is lucid, sometimes the explanations would have been clearer with more supporting numerical examples and diagrams; the treatment of the genetics of

quantitative characters suffers particularly from their absence. There are many points on which the order and method of presentation are nearly the same in the two books, such as the coverage of linkage disequilibrium. On other matters they diverge, as when Crow cheerfully argues for the adequacy of additive models of quantitative characters, while Christiansen and Feldman warn darkly of their drawbacks.

Crow's book may be directly compared with Crow and Kimura's classic *An Introduction to Population Genetics Theory*, published by Harper & Row in 1970 and re-issued by Burgess, Minneapolis, in 1978. It closely parallels the earlier, more detailed exposition in the order of topics and treatment, but the new book, although shorter, is often actually clearer. Sometimes its status as a condensation of Crow and Kimura becomes obvious, however. For example the Poisson distri-

bution is carefully described in an appendix, but never mentioned in the text.

Both Crow and Christiansen and Feldman make the theory clear and accessible, and in view of the need for population geneticists to do more proselytizing I am very glad that these two books are available. But both of them try to present a large body of theory in too brief a space. It may be hard for the reader to understand how the theory relates to real biological problems when the discussion of applications must be short-changed. If the books are used in a course which provides that context, they can do valuable service. If not, then they seem unlikely, of themselves, to bridge the gap between those who ought to apply microevolutionary theory and those who have been developing it to such heights of complexity. □

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Reproductive ways

Daniel J. Schoen

Plant Breeding Systems. By A.J. Richards. *Allen & Unwin*: 1986. Pp.529. Hbk £45, \$75; pbk £19.95, \$34.95.

Quantitative Genetics and Selection in Plant Breeding. By Günter Wricke and W. Eberhard Weber. *Walter De Gruyter*: 1986. Pp.406. DM198, \$79.50.

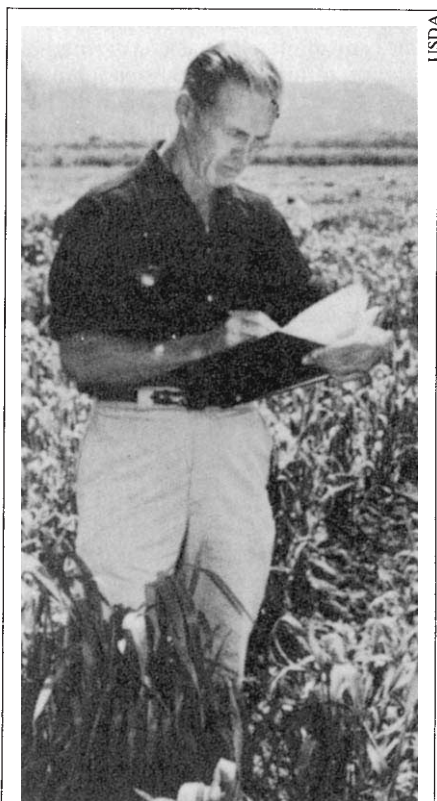
THE manifold ways by which plants reproduce has captured the attention and imagination of evolutionary biologists from many backgrounds. Population geneticists, life-history theoreticians and plant systematists are just a few of the students of the subject. Charles Darwin, not usually remembered for his botanical interests, devoted three volumes to the topic. More recently, a number of theoretically minded biologists working on several continents have collectively enriched the study of why there are so many kinds of plant reproductive strategies by posing a simple question which, in essence, asks: when will a mutation which modifies the sexual expression of a plant (or some other component of the reproductive system) be selectively favoured? The predictions of this relatively new body of theory are often stated in straightforward terms, and are helping to transform the study of plant-breeding-system evolution from a largely descriptive endeavour to an empirically rich and exciting branch of biology.

While Richards acknowledges this progress in his preface, his text is built largely around description of the basic features of plant reproduction systems. The topics include floral morphology, plant-pollinator interactions, gene dispersal, incompatibility systems, gender variation,

self-fertilization and asexual modes of reproduction. Richards is at his best when discussing the classically botanical aspects of the subject. The chapters on floral diversity, self-incompatibility systems in higher plants and asexual modes of reproduction are thorough and useful reviews. Less thorough and precise coverage is given to the genetic aspects of plant breeding systems, such as the estimation of outcrossing rates and the population genetic consequences of different reproductive systems.

My main disappointment with this book concerns the relatively small role which evolutionary theory has played in organizing the subject matter. Further progress in understanding the diversity of plant breeding systems is likely to come as experimentally orientated evolutionary biologists pay close attention to how models of mating system evolution can best be tested, and when theoreticians appreciate the findings and the difficulties which arise when these models are tested. Richards does address the fitness consequences of different modes of reproduction, but he is not explicit as to how these consequences will influence the selection of mating system modifiers. Nor is he clear on how to estimate male and female components of reproductive fitness, which is so essential in examining the processes that are thought to underlie the selection of mating system modifiers. Many of the critical details, questions and experimental approaches required to test plant-breeding system theory are, therefore, largely left up to the reader to devise.

Turning from the ways in which plants mate under natural conditions, to the ways in which plant breeders selectively mate them for crop improvement, Wricke and Weber's book is a solid addition to the recent harvest of new and revised texts



Breeding success — Norman Borlaug, 'father of the green revolution'. The picture is taken from *Plant Science* by John Barden, Gordon Halfacre and Dave Parrish, recently published by McGraw-Hill.

dealing with quantitative genetics. Special consideration is given to practical problems of breeding crop plants with different reproductive and recombination systems. The basic structure of the text (one which I found to be very effective) is separation of the treatments of the quantitative genetic theory of vegetatively propagated, cross-fertilized, self-fertilized and tetraploid crops. Thus, for example, readers with interests in the breeding of cross-fertilized species will find separate sections on the theory of covariances between relatives, the estimation of general combining and synthesizing ability, and mass and family selection, while those interested in self-fertilized species can read separately about the theory and practice of selection with homozygous lines.

The style of presentation is efficient and clean, and the theory is treated in a more quantitative manner than in classic texts such as R.W. Allard's *Principles of Plant Breeding* (Wiley, 1960). The applied nature of the material presented, while not likely to appeal to those teaching quantitative genetics from an evolutionary perspective, makes this a welcome addition to the existing breeding-oriented texts on quantitative genetics. □

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Revolutionary zeal

Daniel H. Janzen

Plant Ecology. Edited by Michael J. Crawley. *Blackwell Scientific*: 1986. Pp.496. Hbk £35, \$55; pbk £16.50, \$29.95.

MICHAEL Crawley set out to put together a plant ecology text for the undergraduate, a text that would haul itself out of the musty bog of old world descriptive and aggregate plant ecology and into the chaos of new world evolutionary and ecological biology of plants as individuals and populations. He was generally successful. However, about half of the (very competent) contributors did not fully share or appreciate Crawley's legitimate zeal for this revolution in the way plant ecology is taught in English universities. Many seem to have taken the task on as an "assignment" or as a chance to get a fat review manuscript into print. Were I an undergraduate discovering plant ecology through this book, I think I would be mostly bored. Give the Crawleys of the world the time and resources to write such texts straight through, with a pedagogical, as well as a factual, goal in mind.

The book is built on summary statements, with occasional examples. However, the authors are sometimes not familiar enough with the biology of the examples to avoid over-generalizing. After being told that in animal ecology "the individuals are assumed to blunder about at random", we are informed that "plants compete strongly with only the six or so individuals in their immediate vicinity" (p.253) — someone forgot about competition for pollinators and dispersal agents. And we are told that "strictly speaking, no animals are adapted for pollination" (p.187), when hundreds of species of fig wasp (Agaonidae) are unambiguously adapted for pollination of figs, by any definition of either "adapted" or "pollination".

Again, it is stated that "theory predicts that maximal species richness should occur in moderately resource-poor habitats, habitats that have just enough resources for species survival in the absence of interspecific competition" (p.59). Such a theory is incompatible with the decreasing gradients in plant species richness up the sides of tropical mountains. An entire chapter is based on the concept that "factors causing major reproductive deficits or imposing significant mortality are bound to have evolutionary consequences when there is genotype variation in their effects" (p.336). It would seem that both disruptive selection and phylogenetic inertia were forgotten.

The evolutionary philosophy running through the book is that where there is a selective pressure it is responded to; plant

traits are maintained through selection; and those selective pressures are present in the environment of the plant today. All three suppositions are highly suspect in many aspects of field biology. As the preface notes, "Throughout the book we are at pains to stress that our ultimate objective is to measure the impact of the processes we describe on the *fitness* of individual phenotypes" (p.xi). Given that objective, the book would have benefited from an introductory chapter examining why or whether the concept of fitness should be used as the primary point of reference for the plant's interactions with the world. A single phenotype may have extraordinarily different fitnesses in the variety of habitats it occupies and continues to occupy

generation after generation. Even when variation appears in the genetic background for that single phenotype, the result *need* not be any realized selection or evolution (though of course it may be — p.251).

Plant Ecology is not horrendously long, and will certainly serve as a good foil for an alert teacher working with well-motivated students. But this kind of teaching, so common in English universities, is rarer in the United States. That leads me to worry about how the book might be used indiscriminately in the New World. □

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Flying and fishing

J. Bryan Nelson

Seabird Ecology. By R.W. Furness and P. Monaghan. *Blackie/Chapman & Hall*: 1986. Pp.164. Hbk £19.95, \$45; pbk £9.95, \$23.

FURNESS and Monaghan begin their book with an exhilarating, free-style swim through the seas of seabird ecology. Mating systems, colonial breeding, deferred breeding, clutch and brood-size and much else are polished off in a mere 19 pages. It is a tribute to the authors' lucid style and grasp of their material that they nevertheless convey the essentials of the subject (it was presumably a slip to exclude the Suliidae from the families of the Pelecaniformes in the first table). I would have welcomed fuller discussion of social behaviour in relation to breeding ecology, more on the assessment of mate quality (what is the evidence that courtship displays are to assess mate quality?) and something on synchrony, the phenomenon of intra-specific interference in breeding colonies and on non-breeders. But one can't have everything in a short account.

At the core of the book are the twin themes of seabird feeding and the interactions of man and seabirds. Almost one third of the contents is devoted to seabirds and fisheries. This selectivity provides a richer texture to the book than a uniform précis, though one does notice the change in writing style from that appropriate to a scientific paper to that used in textbooks. As an adjunct to the emphasis on food and fisheries the authors devote much space to bioenergetics. This, too, is defensible, for this relatively new field generates important computations of the amount of energy which seabird populations take out of the marine environment. Throughout, however, the authors are duly mindful of the pitfalls of using these equation-generated quantities to predict fishery

matters. Marine systems are highly complex, involving many interacting species at different trophic levels, and the changes which occur in them will seldom be even qualitatively predictable.

The short review of theories of population regulation is admirable and comes down, in the main, on the side of density-dependent regulation (Wynne-Edwards's new book on group-selection appeared too recently to be mentioned). I myself believe that the regulation of many seabird populations is not density dependent, and the authors, too, include appropriate reservations. Both with regard to population regulation and to the interactions of fishermen and seabirds, the Peruvian anchovy fishery receives much attention. Here I failed to understand how the numbers of the birds concerned could have been unregulated a century ago (p. 45). Niño years have been occurring for much longer than this and were in themselves regulatory. Only once do the authors appear to claim (p.79) that the increase in Peruvian seabirds following a huge, natural reduction was due to the decreased competition for food. Surely, until the relatively recent overfishing by man, the seabirds climbed rapidly back to high numbers because food, irrespective of seabird demands on it, was superabundant?

The section on monitoring the marine environment is timely and comprehensive, dealing with plastics (ingested by seabirds), oil, PCBs, organochlorines and heavy metals. The technique which allows the estimation of mercury levels in feathers could be especially useful because, when extended to museum specimens, it makes historical comparisons possible.

I enjoyed and learnt much from this excellent book. Students, and indeed their teachers, as well as fishery scientists and serious seabird buffs should go out and buy it. □

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Lost in translation

John Eaton

Handbook of Limnology. By J. Schwoerbel. Ellis Horwood: 1987. Pp.228. £29.50, \$53.40.

SCHWOERBEL's *Handbuch* has been established as a standard text in many European universities since the first edition appeared in 1971. Limnology is treated in its broad European sense, to include flowing waters as well as lakes. This translation of the fifth edition of 1984 is therefore a potentially useful addition to the range of textbooks available to English-speaking students of freshwater biology.

The book has distinctive emphases on the historical development of the subject and on the influence of catchment on the ecology of inland waters. The examples used draw heavily on the German literature, but although the coverage could be criticized as parochial in this respect, it is no more so than in the many American texts which almost wholly ignore the immense European contribution to limnology. More serious limitations are the lack of tropical studies and the small extent to which literature sources have been brought up to date. For example, in palaeolimnology the most recent citation

is 1974, so none of the advances in techniques and knowledge of the subsequent decade are mentioned.

However the worst failing is the poor-ness of the translation. German sentence construction is often retained, producing a tortuous English style. There are inaccuracies (for example, on p.109 the dinoflagellate *Peridinium* is said to have cilia) and obscurities abound (as on p.27 where resistances to movement through water are translated as "pondering effects"). Together with the frequent misprints, the result is such baffling sentences as the one on p.18, on sources of rivers: "In their terminal budget as well as their colonisation there are large differences between these innological (not hydrological) source categories".

It is a pity that such a potentially valuable book should be spoilt by such easily remediable faults. The *Handbuch* contains fascinating insights and syntheses of information, but the difficulties in understanding this translation will deter many of the students who presumably constitute its main intended readership. Indeed it is hard to see any obvious market for this book, as water engineers and environmental consultants will continue to turn to more advanced texts. Here is an opportunity sadly missed, and a volume that is a marked contrast to some of the other, excellent titles that have been produced by this publisher. □

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Sea sicknesses

A. Nelson-Smith

Marine Pollution. By R.B. Clark. Clarendon: 1986. Pp.215. Hbk £19.50, \$35; pbk £9.95, \$17.95.

THERE is an enormous, widely scattered and often obscure literature on marine pollution, much of it hard to get hold of. There is, then, a need for a broad but succinct account of the subject; as editor of *Marine Pollution Bulletin* and member of several distinguished British or UN expert groups, Professor Clark is well placed to write such a book.

My review copy of *Marine Pollution* is a solidly bound paperback, only slightly larger than pocket-size and as comfortably priced. The book adequately covers the field implied by its title, with a selection of classical and contemporary examples, and opens with a consideration of the nature of pollution. Successive chapters work through the main groups of pollutant, not omitting heat, munitions, plastic waste and dredging spoil. There follows description of the state of five more-or-less enclosed seas of particular concern and, finally, discussion of the problems of assessing pollution impact.

In the interests of brevity inessential details have been omitted and explanations simplified, but the result still meets the needs of undergraduates whilst remaining comprehensible to the lay reader. The author fails to deal with a few basic points (why mention chemical oxygen demand without explaining the significance of its difference from its biological counterpart) and gives too brief an account of others (eutrophication, for example). There is, too, some unnecessary material: an account of the nature of radioactivity in less than two pages, including diagrams of atomic structure, is surely inadequate when it takes much the same amount of

solid text to explain the 'critical pathway' approach when setting permitted levels for radioactive discharges. Similar inequalities of treatment are seen elsewhere—a diagram comparing three slightly different types of 'slick-licker' (used in cleaning up spilt oil) occupies most of one page, but no mention is made of the many designs of skimmer, nor of sea-booms, bubble-barriers and oil-herding chemicals that are used for containing or deflecting spills.

It is difficult for natural scientists to avoid a tone of righteous indignation when discussing pollution. Here the author succeeds admirably in maintaining the book's balance by emphasizing that marine ecosystems have a great capacity for recovery from damage; even a permanent change in component species need not necessarily be a bad thing. There is also a reminder that an improvement in effluent quality of greater than 70–80 per cent is usually impracticable, and that most pollution is in any case produced either by us or for us—a point that many armchair environmentalists conveniently ignore.

In place of a bibliography, eleven books or reports (each with extensive references) are listed as further reading. In addition, the sources of figures and tables are given as abbreviated literature citations. This, together with a comprehensive index, confirms the academic rather than popular flavour of the book, which is one I shall certainly be urging my students to read. □

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• *Earth*, by Anne and Paul Ehrlich, a highly illustrated "summary of Earth's environmental predicament", was published in Britain last week; the appearance of the book coincides with a three-part series, presented by the Ehrlichs, running on independent television. Publisher is Methuen, price is £14.95.

Philosophy for the nervous student

Michael Ruse

Investigating the Life Sciences: An Introduction to the Philosophy of Science. By Geert M.N. Verschuuren. Pergamon: 1986. Pp.148. £20.95, \$30.

THIS slim volume introduces some of the basic concepts in the philosophy of science, and then develops the ideas by looking at biology — both historically and as it is today. There are four parts. The author starts with a general introduction, talking about the use (and misuse) of words, the nature of facts and whether there can ever be facts without theories, the relationship between our thought and the external world (if there be such) and so forth. Then, second, there are several brief tales about important episodes in the history of the life sciences: Harvey on the heart, Mendel on heredity, Darwin on evolution and the like.

Verschuuren then turns to some problems in contemporary biology. He is fairly conservative on the nature of science and (even more so) on its practitioners. The old distinction between the context of discovery and the context of justification is trotted out, and treated as if it were unproblematical. Nor, for instance, is the reader weighed down unduly by a major controversy going on at the moment (particularly between philosophers interested in the life sciences), about whether or not the aim in science is to find universal laws, or whether it is to find limited models

which hold for one or two cases but not generally.

Finally, we get to the 'limits of science' and such issues as reductionism and determinism. My jaw dropped a little at the end, where the author states that all scientific activity since the Reformation has made little difference to our essential thinking about ethics and religion. It is difficult to believe that the fact that we are modified monkeys, contingently the end product of a long, slow, blind process of evolution, rather than a favoured miraculous Creation of a good God some 6,000 years ago, makes no difference to our religious beliefs — or to our views on right and wrong.

It seems to me that *Investigating the Life Sciences* is a book written by a teacher rather than a scholar. That sounds insufferably condescending but it is not meant in a flatly negative way. The book contains no contribution to the subject, nor (and this more of a criticism) is there any real acquaintance with much that is new in philosophy of science (in general) or philosophy of biology (in particular). But the author has a happy knack of laying out positions quickly and clearly; he does not bog down the reader with too many details — hardly possible anyway in a book of this length; and he has a real gift for setting down an idea diagrammatically, separating out concepts with a few strokes of the pen.

I doubt if I would use this book as a text on a course. But I might — and probably will — loan it to a nervous student who needs a friendly guide into the subject. □

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Of DNA and RNA

Peter Piper

The Biochemistry of the Nucleic Acids, 10th Edn. By Roger L.P. Adams, John T. Knowler and David P. Leader. Chapman & Hall/Methuen: 1986. Pp.526. Hbk £35, \$59.95; pbk £16.95, \$33.

THIS text is now in its second generation of authors, which is the reason why editions of scientific works, unlike certain musical enterprises, do not have to end with the ninth. Previous versions of the book were, in their time, well received as concise reference sources on nucleic acid biochemistry. All of the chapters in this latest edition have been updated to the end of 1985. With its clear style of presentation, informative diagrams and extensive bibliography it will be welcomed by final-year undergraduates, and will also be used widely for reference by molecular biologists.

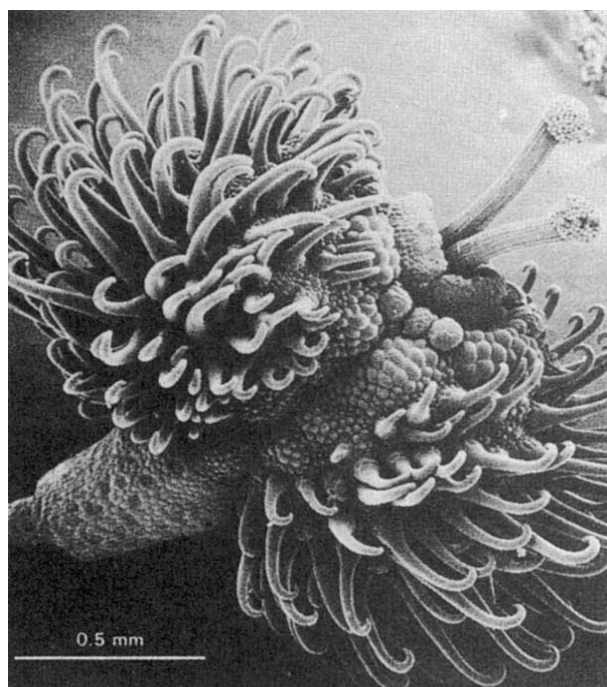
As the title implies, the stress is on both the structures of nucleic acids as well as the enzymes and ancillary proteins that interact with them. On the structural side, chromosomes and the ribosome are discussed in detail with an eye to their function. On the enzymological side there are lucid accounts of the metabolism of nucleic acid precursors, of DNA and RNA synthesis and maturation, of viral replication and of DNA rearrangement. Especially helpful are the various tabulations of enzyme activities, and the correlations of these activities where appropriate to the products of specific genes.

With its emphasis on the biochemistry that has led to so much of our knowledge of nucleic acids, this text is a valuable complement to other works that concentrate on the contribution made by more genetic approaches. It is generally appreciated that topics such as the enzymes involved in DNA replication, repair and recombination, the mechanisms and possible roles of RNA splicing or the actions of transcriptional control elements can no longer be given adequate treatment in those standard volumes that aim to cover the whole of biochemistry. There is therefore an increasing need for well-balanced accounts of these and similar areas, a need that this book satisfies very successfully.

This is not a text to consult for details of laboratory methods. Apparently in apology for the fact that biochemistry is an experimental subject, there is a final chapter on methods of studying nucleic acids. This is a highly readable, if succinct, introduction to the experimental side of nucleic acids and cloning. □

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Hitch-hiking apparatus — scanning electron micrograph of a goosegrass flower showing two developing fruits and the hooks by which, through attachment to passing animals, the fruits are dispersed. The picture is reproduced from the third edition of Karen Arms and Pamela S. Camp's Biology, published by Saunders. The book appeared too recently to be included in the review by Cox and May on p. 225.



Biophoto Associates

Fresh transmission

Peter J. Roberts

Chemical Neurobiology: An Introduction to Neurochemistry. By H.F. Bradford. W.H. Freeman: 1986. Pp. 507. \$36.95, £21.95.

AT THE research level neurochemistry is well established as a distinct discipline. In teaching, however, it is treated as a rather hybrid creature, various aspects being covered in courses on biochemistry, pharmacology, biology and medicine. It is therefore not surprising that hitherto there has been no integrated text on the subject at a level appropriate for undergraduates. Professor Bradford's book neatly fills the gap, and with its comprehensive listings of other relevant publications will also be of value to postgraduate students.

In his preface, the author points out the importance for neurochemists of a thorough grasp of neuroanatomy. Wisely, however, he does not attempt to provide a biochemist's introduction to the topic. An appendix provides suggestions of appropriate core texts and learning programmes, while the early part of the book describes in adequate detail the structural organization of neurons and glia, specific markers for these cell types, and their functional interactions. Energy metabolism (both *in vivo* and *in vitro*) is surveyed rather briefly, though it is fair to argue that the principles are little different from those investigated in, say, liver, and that it is justifiable to concentrate on the uniqueness of the nervous system. Non-invasive imaging techniques are also touched upon (2-deoxyglucose, PET scanning, NMR and so on), but the student is unlikely to gain much of a feel for the power and limitations of these approaches.

The emphasis of the book, as one would expect, reflects the author's interest in synaptic transmitter function. All of the main systems and their associated ionic and second messenger systems are considered, but this is not yet another book (amongst many) on transmitters — on the whole I found these sections appropriate and refreshing to read. The biochemical events underlying transmitter function are stressed, although this is put into proper context by discussing localization of pathways. The subsections on the pharmacology of the various receptor systems are rather less satisfactory. Some of this material is confusing and students, I think, would feel the urge to reach for an appropriate neuropharmacology textbook.

One of the first words that neurochemistry students learn is 'synaptosome', and Professor Bradford puts across the importance of these structures as working *in vitro* models of synaptic function with

enthusiasm and authority. After discussing synaptic structure, metabolism and transmitter dynamics, he then outlines experimental uses of the preparations in relation to, for example, neurotransmitter interactions and dissection of the synaptic apparatus. The student will quickly realize what a vast amount of basic information has accrued from such reductionist approaches to the brain.

The last two chapters diverge from basic neurochemistry. One deals with central neurotransmitter systems and behaviour (behavioural pharmacology) — this area is still very much a minefield, and although certainly of interest to the student I feel that inclusion of discussion of the subject was a mistake. The other chap-

ter contains a good description of transmitter dysfunctions in the classical neurological and neuropsychiatric disorders. This does not add much to the many other chapters in other accounts of this topic, but when viewed as part of the book as a whole it fits well and again is well referenced with up-to-date papers and reviews.

Chemical Neurobiology is uniformly well written, and there are many clear micrographs and informative figures. The production, too, is of a high standard and the layout is easy on the eye. The book is bound to be popular, and deservedly so. □

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Learned pursuits

Geoffrey Hall

Introduction to Comparative Cognition.

By Herbert L. Roitblat. W. H. Freeman: 1986. Pp. 377. Hbk \$29.95, £29.95; pbk \$17.95, £17.95.

ONE of the main aims of the experimental study of learning in laboratory animals has been to build the foundations of a general theory for all behaviour. The rat in the maze and the pigeon in the Skinner box have been seen as model preparations from which the psychologist might conveniently derive general principles in much the same way, say, as the geneticist makes use of *Drosophila*. And just as the geneticist's laws of heredity have formed the basis of an understanding of human patterns of inheritance, so, it was hoped, studies of animal learning might help us to understand human cognition. Broadbent's seminal book *Perception and Communication* (Pergamon, 1958), the starting point for modern cognitive psychology, drew upon theories developed by students of animal learning.

Now the tables are turned. Animal-learning theorists have failed to produce a widely accepted general theory, and have watched with some envy the apparently rapid progress being made in the study of human information-processing. Some of them, and Roitblat was one of the leaders of the trend, have adopted concepts from human cognitive psychology (in particular, theories of working and reference memory, and the notion that environmental events engender mental representations that are subject to further processing) in their analysis of animal learning. Roitblat has now made the first attempt to provide a textbook, appropriate for advanced undergraduates, summarizing the new approach, and on the whole he has been successful. The material is well organized, the style is clear and every effort

has been made to render intelligible the complex experimental designs that this approach to animal learning often gives rise to. The chapters reviewing studies of working memory and the sensitivity of animals to time and serial order, topics that Roitblat clearly sees as lying at the heart of his subject, are particularly useful.

A couple of doubts remain, one concerning presentation and one more fundamental. First, Roitblat presents comparative cognition as constituting a paradigm shift, a scientific revolution in which the earlier behaviourist approach is overthrown. Surely this is too large a claim. As the author is aware, many of the cognitive concepts he espouses have been employed without fuss and for some time by workers in the behaviourist tradition — indeed, one of the strengths of the book is the way in which it integrates earlier work on associative learning and conditioning with more recent studies of animal memory.

The second point concerns the relationship between cognitive processes in humans and non-humans. Many have argued that our species, perhaps by virtue of its language, possesses a set of cognitive mechanisms that other species lack. If this is so, then an attempt to account for animal cognition using theories and concepts derived largely from studying the ways in which humans process verbal material is unlikely to make much progress. Roitblat gives us a brief survey of the attempts that have been made to teach some version of a human language to apes and to a few other species, but fails to deal with the central issue directly.

Perhaps the proof of the pudding is in the eating, however: if the next few years see the fulfilment of the promise of this book — the development of a unified account of cognition in animals and man — then the doubts of the sceptics will be laid to rest. □

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Problems of living together

D.H. Jennings

Symbiosis: An Introduction to Biological Associations. By Vernon Ahmadjian and Surindar Paracer. *University Press of New England*: 1986. Pp.212. \$32.50, £26.90.

The Biology of Symbiosis. By D.C. Smith and A.E. Douglas. *Edward Arnold*: 1987. Pp.302. Pbk £16.95, \$29.95.

IN THE 1960s, the Biological Sciences Curriculum Study — that important American initiative in school biology — showed that biology could be approached starting off with the cell, the organism or the ecosystem. Today one might begin with symbiosis, the permanent or long-lasting association between two or more species. If that approach to biology were to be adopted at university level, these two books would be standard reading. In their different ways they are excellent introductions to the subject, demonstrating why investigation of the often intricate ways in which a symbiotic association may function is such a fascinating exercise and that it is open to contributions from anyone, whether they be the patient observer or the molecular biological whizz kid. And although we may here be speaking of textbooks, both can be read with profit by all professional biologists whatever their background. Photosynthesis, immunology, *r* and *K* selection and many other aspects of modern biology are all touched upon, but from the unique viewpoint of symbiosis.

Ahmadjian and Paracer are all-embracing in their approach. Rightly taking the definition above as provided by de Bary, they survey associations involving all organisms from viruses through bacteria, to unicellular eukaryotes and multicellular organisms such as helminths and mistletoes. The style is succinct, but perhaps a little breathless, and this is not a book to be read continuously. Nevertheless a student will gain much information from a chosen chapter or section. Though there are excellent half-tone figures, the diagrams leave something to be desired and a few will cause confusion among the uninitiated. But what the book lacks above all, perhaps, is a real feeling for the experimental challenge in probing a symbiotic association.

It is this particular theme that runs through Smith and Douglas's text and makes it so stimulating. Their account is more circumscribed than that of Ahmadjian and Paracer, dealing with what can be called mutualistic associations involving very intimate contact between the partners, one of which is a micro-organism, such as algae in protists, corals and

lichens, mycorrhizal fungi and the rumen microflora. But the authors do marvellous justice to such matters as how we can quantify benefit or how we can identify the recognition systems involved. Numerical information is used extensively, there are good half-tones and the diagrams are models of how to convey information in an uncluttered manner. While one can grumble about the title, which is somewhat misleading, the book sets out much of the experimental evidence that underpins the symbiotic theory of the origin of the eukaryotic cell and provides valuable in-

sights into microbial ecology as it relates to the host organism as a habitat.

Where both sets of authors fail somewhat is in their overviews at the end, in that neither grapples with the central issue of self and non-self. I suspect, however, that some other text is required to do that properly, and it is a measure of the success of these two books that they provide the foundation for a better consideration of the broader issues. □

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Biology writ large

Edward C. Cox and Robert M. May

Biology. By Peter H. Raven and George B. Johnson. *Times-Mirror-Mosby/Blackwell Scientific*: 1986. Pp. 1,198+. \$38.95, £27.50.

Biology. By James M. Barrett, Peter Abramoff, A. Krishna Kumaran and William F. Millington. *Prentice-Hall*: 1986. Pp. 1,167. \$42.65.

Biological Science, 4th Edn. By William T. Keeton and James L. Gould. *W.W. Norton*: 1986. Pp. 1,175. Hbk \$36.95; pbk £19.95.

The World of Biology. By Thomas G. Overmire. *Wiley*: 1986. Pp. 557. \$29.25, £28.30.

THE competition among introductory biology texts over the past ten years and more provides an off-beat example of what evolutionary biologists call Cope's Law — a tendency to ever-increasing body size. Indeed, conventional bibliographical details in book reviews omit what to many students may come to be the most important parameter characterizing these books: Raven and Johnson weighs 3.4 kg, Keeton and Gould 2.8 kg and Barrett *et al.* 2.9 kg. It may be time for publishers to start thinking of issuing textbooks as a collection of separate volumes, or else to bind them in carrying straps or even on wheels.

This trend towards giantism derives from our growing understanding of the biological world, at every level from molecules to ecosystems. Publishers and authors presumably believe — probably correctly — that this expanding body of knowledge has to be surveyed fully, lest a competing text be seen as more comprehensive or lest a lecturer's own interests be omitted. It would be nice to see an attempt to present introductory biology in a more digestible way that focused on a few central themes (as distinct from striving after relative brevity by reducing the ocean of facts to a vast, shallow lagoon of them). Such a text would, however, probably be seen as idiosyncratic.

Another conspicuous tendency is the increasing excellence of the illustrations in introductory biology texts, as competitors strive to match the high standards originally set by Curtis. It makes the present books a pleasure to thumb through, although there is a danger that pedagogic purpose may occasionally be subordinated to illumination of the page.

Barrett *et al.*, and Raven and Johnson, are both new books. Raven and Johnson, indeed, explain at the outset that their book is based on extensive intellectual and marketing surveys, and they scold other authors for perpetuating inaccuracies and for cribbing from each other. The organization of the book is interestingly different from the usual molecules-through-cells-to-organisms: it begins with sections on the origins of living things, the biology of the cell, genetics and heredity, and evolution; it then moves on to the biology of simple organisms, of plants, of invertebrate animals and of vertebrates, and concludes with ecology. This reorganization works well. Such fundamental rethinking has had some concomitant 'oopses'. Immunology, for example, is left out entirely; a supplementary chapter on the subject is now available, and will be bound into later editions. And there are several errors, although these are corrected in a later list of errata which has been mailed out. For instance, those familiar with the history of ideas about altruism and kin selection will be interested to read an account of "a passing remark" by Haldane (no mention of Hamilton anywhere), to the effect that he would lay down his life for two brothers or four first cousins. This error (it should be eight first cousins) is reiterated in a problem, to which the wrong answer is given ("would you lay down your life for one brother and three first cousins?"). Not surprisingly — Raven is a distinguished ecologist — the ecology section is good, and an evolutionary perspective suffuses the text in a way we like very much.

The book by Barrett *et al.* is organized along more conventional lines, little-to-big. It begins with a thoughtful discussion of what science can and cannot be, what its rules are, why it is not — and perhaps

never can be — complete, and how it can benefit society yet why that cannot be the only measure of success. This book is better written than most, with the right pace, a clear and direct style, good use of language, and excellent connections between text and illustrations. First-rate essays (set as what the authors call “panels”) enliven the accurate text. Like most introductory biology texts, however, this book lacks the pervasive evolutionary perspective of Raven and Johnson.

Jim Gould has produced a thorough revision of Keeton, which (with Curtis) has led the field in recent years. Gould is a colleague of ours, and conflict of interests forbids us from more than briefly mentioning this book. We would, however, urge teachers of introductory biology to read it, and to examine the comprehensive set of accompanying material (laboratory

guide, study guide, teacher's manual, sample tests).

Overmire's *The World of Biology* is a shorter and simpler book, aiming to contend with the reduced versions of Arms and Camp or Keeton for adoption in more elementary survey courses. It is conventionally organized, accurate and ‘simply written’ in a way that, although clear, makes minimal assumptions about the vocabulary of the reader. Overmire manages to convey a real sense of concern for current environmental problems, without forcing opinions down the reader's throat. The illustrations are good, and well integrated with the text, although not up to the extraordinary quality of the three big books. □

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Life's processes

Ole H. Petersen

Physiology and the Scientific Method: The Design of Experiments in Physiology. By Evelyn M. Scott and James M. Waterhouse. *Manchester University Press/Longwood, Wolfeboro, New Hampshire*: 1986. Pp.204. Hbk £20, \$34.50; pbk £8, \$14.

Principles of Physiological Measurement. By James N. Cameron. *Academic*: 1986. Pp.278. Hbk £49.50, \$59.50; pbk £20, \$23.95.

Lecture Notes on Human Physiology. Edited by John J. Bray, Patricia A. Cragg, Anthony D.C. Macknight, Roland G. Mills and Douglas W. Taylor. *Blackwell Scientific*: 1986. Pp.616. Pbk £9.95, \$19.50.

IS THERE anything useful to be said about *physiology* and the scientific method? Having read Scott and Waterhouse's book with this title one is tempted to give the answer ‘no’ and to recall Popper's statement that “We are not students of some subject matter but students of problems”. The less ambitious subtitle is *The Design of Experiments in Physiology*, and the book is in fact intended as a practical guide to experimental work for final-year physiology students.

The first part deals with such matters as choosing species and types of preparation, experimental design, healthiness of preparations, measurements and recordings,

presentation and statistical assessment of results, and finally with drawing conclusions. The second part consists of six ‘abstracts’ based loosely on published papers. Each of these abstracts is a very brief version of a scientific article followed by a number of questions and suggested answers, all intended to illustrate some of the general principles set out in the first part of the book. The authors are very keen on the ‘physiological normality’ of experimental preparations, but seem unaware that a great many important experimental results have been obtained, and could only be obtained, under conditions that are very far from normal. In all, the book is superficial, and because of the arbitrary selection of material will not meet the needs of most students.

Another ‘general principles’ book, by James Cameron, deals with physiological measurements. The aim has been to provide a “compact reference for the commoner methods in use in physiology as an entry point for the student without such exposure”. In a short volume such as this the main problem is the selection of material. Overall, gas analysis, which seems to be the main interest of the authors, dominates the contents. But although this is an important topic, it does not justify the general title of the book. There are also chapters on radioactive tracers and some basic ones on gas and solution concepts, electronics and transducers, but, astonishingly, none on electrophysiology. It is very hard to think of a physiology course that contains no neurophysiology, cardiac electrophysiology or membrane physiology; indeed, these topics would dominate many courses, and the fact that this book has nothing to say about extra- or intracellular electrical recordings, not to mention the increasingly important area of single-channel current recordings, is a major defect.

This is a pity, for some of the sections,

for example that on pipetting, are excellent with many helpful practical details that are difficult to find elsewhere. In the chapter on pH measurements, however, important advice concerning calibration is missing. Many laboratories are unaware of errors arising from liquid junction potentials when pH meters with the commonly used combination electrode are employed. The point should have been made that pH calibration needs to be done using salt concentrations relevant to the test conditions in order to avoid errors which can be as large as half a pH unit.

The modestly titled *Lecture Notes on Human Physiology*, edited by a group at the University of Otago Medical School, is a short but comprehensive textbook of physiology, well suited to most courses for medical students. Of the many shorter physiology texts that I have reviewed in recent years this is by far the best. It is written with great clarity, using simple but precise diagrams of uniform style, and contains virtually no important errors. It is a pleasure, for once, to see a correct description of the relationship between aortic and left ventricular pressures, and to have good explanations of both cardiovascular and respiratory changes in the transition from rest to exercise.

Equally impressive is the chapter on kidney, water and electrolytes, which is clear and up to date, and contains more precise information than is found in many larger textbooks. Having previously complained about the lack of discussion of osmotic diuresis in several other texts, I am glad to see that this point is well explained here. Inevitably there are some weaknesses, and the chapters on endocrinology and gastrointestinal function stand out as being less complete and up to date than the others.

It often takes a long time for new concepts to be incorporated into textbooks, and in physiology the situation seems to be worse than in many other subjects. In *Lecture Notes on Human Physiology* it is disappointing to find that although the patch-clamp method for single-channel current recording is introduced with a schematic diagram, virtually no use is made of single-channel information in the discussions of electrical activity in nerve, muscle and other cells. This is a mistake, because a molecular approach to membrane physiology makes it much easier for students to understand basic ideas in this field. Now that physiology has its own short review journal, *News in Physiological Sciences*, we must hope that textbook authors will find it easier to keep up with developments and will take the trouble to integrate novel concepts into the main text when these serve to clarify the fundamentals of the subject. □

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• Also published last year, by Raven, was a second edition of *Respiratory Physiology* by Allan H. Mines. The book has been up-dated generally, and new topics included, and like its predecessor is intended mainly as a course textbook for students of medicine and allied subjects. Price is pbk \$19.50.

Going through the motions

Judith Armitage

Cell Movement and Cell Behaviour. By J. M. Lackie. *Allen & Unwin*: 1986. Pp. 316. Hbk £30, \$45; pbk £11.95, \$19.95.

As ITS title declares, this book undertakes to cover all of cell movement and its control, from muscle to amoeba, from mitosis to bacterial flagellar rotation. The author admits in his preface that no one person can know closely more than a small part of this now-huge field of research, so from the outset he set himself a very difficult task. I understand, however, that there was also an inordinate delay between the submission of the typescript to the publisher and its appearance in printed form. The result is a book which might have been exceptional, but as it can only be recommended with reservations.

Dr Lackie is an expert in leukocyte movement, and in dealing with this subject his references are up to date and the writing flows with ease. This readable style overflows into the accounts of other areas, for example those on bacterial motility and chemotaxis. Here, however, arguments are put forward for cyclic GMP being the sensory signal, an idea now all but abandoned; also gene names are used and gene product roles are assigned that have been out of date for several years. Similarly, it is unfortunate to see a chapter in a book published in August 1986 beginning with the statement that "The association of microtubules with movement is, with one important exception, rather more an article of faith than a well documented reality..." — new techniques turned that faith into reality some time ago. These points may seem to be not particularly important, but misinformation of this sort will confuse the advanced students taking courses on immunology, pathology and cell biology at whom this book is aimed.

To be fair, the sections I have criticized are a small part of the book as a whole. Because of Dr Lackie's imaginative approach, the rest of it will provide students with an illuminating view of the subject. There are three main sections. They deal with different types of motor; with how these are used by different cells to move, including clear explanations of why each is suited to a specific task; and with the differences in the use and control of these mechanisms in uniform and anisotropic environments. Particularly good are the introductions at the beginning of each section, where Dr Lackie explains by analogy and with simple diagrams the problems of size, shape, surface and gradient faced by single cells, and really gets the

reader to think down to the size of the individual cell.

The book is illustrated with well-planned diagrams to support the text and with plenty of electron and light micrographs. Again, however, in the accounts of bacterial motility and microtubules many of the figures should have been updated.

Overall this is a highly readable and digestible book which will be very useful to students of eukaryotic cell biology. But anyone reading it should take the bacterial and microtubule sections as historical rather than providing a current view of the field. □

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Sole contributions

John Edwards

The World of the Cell. By Wayne M. Becker. *Benjamin/Cummings*: 1986. Pp. 882. \$39.95, £36.95.

Molecular Cell Biology. By Charlotte J. Avers. *Addison-Wesley*: 1986. Pp. 704. \$19.95, £19.95.

Genetics & Molecular Biology. By Robert Schleif. *Addison-Wesley*: 1986. Pp. 626. \$20.95, £20.95.

Molecular Biology, 2nd Edn. By David Freifelder. *Jones & Bartlett*: 1987. Pp. 834. \$38.75.

THE ever-expanding synthesis of cell and molecular biology has been celebrated in two outstanding multi-author textbooks: *Molecular Biology of the Cell* by Alberts *et al.* and, more recently, *Molecular Cell Biology* by Darnell *et al.*

Single authors of new texts will argue that for some levels or periods of study these volumes on the one hand may overwhelm with their sheer scale or, on the other, sacrifice depth for breadth. But those who set out single-handed to tackle the broad canvas can know only a small part of the relevant primary literature. This may not matter too much for presenting neat accounts of the latest models, but the how and why inevitably suffer.

Another result can be the propagation, particularly among peripheral material, of convenient oversimplifications, curious choices of example or even downright myths. For the first, see the description of Frye and Edidin's antigen-mixing experiment in the books by Avers and Becker (both label and fuse in the wrong order). For the second, the Alberts-like inclusion of a sponge aggregation factor and omission of the well-characterized vertebrate cell adhesion molecules in Avers. For a myth, that transformed cells in culture

have lost contact inhibition of locomotion (Avers) or, worse, that this loss causes them to grow to higher densities (Becker).

Becker's sweep is broad, "a comprehensive introduction", from biochemistry (with the emphasis on energy), through organelles and molecular genetics, to development, motility, the nervous and immune systems, and cancer. Of the problems that follow each chapter, some simply check if the reader stayed awake. Others are imaginative and entertaining, and together with some jolly analogies and an occasionally breezy style show an author working hard to make the material vivid for students.

I am not convinced, however, that Becker succeeds very well in his declared aim "to explain not only what we know, but also how". For example, the account of muscle contraction features the cross-bridge cycle, but apart from a fragment of evidence in the problems the only experimental source hinted at is electron microscopy. Here and elsewhere I would have preferred to see a few more comments such as "it is thought that" or "one idea is", rather than the stone-tablet style. Despite trumpeting for the cytoskeleton, microfilament bundles and tissue cell locomotion are omitted. The material on tumour cells is weak. I fear the experiment on cell sorting is imaginary. The choice of images of fluorescent microtubules to illustrate effects of transformation on the cytoskeleton is unfortunate, because leading practitioners are on the record as stating that this effect is an optical artefact. And a curious omission from the essay on *ras* is mention of the point that p21 has hydrolytic activity towards the GTP it binds, and that this activity is decreased by the oncogenic mutations. For a book at this level I prefer Karp's *Cell Biology*, reviewed here in 1985.

Charlotte Avers's *Molecular Cell Biology* is perhaps one-third the length of the multi-author work of the same name. The subset of material she has chosen has the intact cell as the upper limit rather than the centre, and the emphasis is strongly eukaryotic. Chapters on cell surfaces, organelles and genome are followed by "Reproduction and Development" (the cell cycle, mitosis and meiosis, rather than a foray into embryology), and cellular and molecular evolution. There are flaws. In the introductory biochemistry, should not the idea of structures being favoured by hydrophobic clustering appear in relation to protein conformation as well as membranes? In two figures, hyperbolic curves are shown reaching their asymptotes when they should still have some way to go. Later, decamin is said to be a different intermediate filament protein of fibroblasts, but it was in fact an earlier name given to the pair now usually known as vimentin and desmin.

The core of the work, however, is

admirably done. Here Avers manages concisely and thoughtfully to indicate historical perspectives, some of the key experimental evidence and, most importantly, the existence of areas of ignorance and of more than one point of view on a number of issues.

I had not met David Freifelder's *Molecular Biology* in its first edition, but have enjoyed dipping into the second. This is very much molecular biology in its traditional territory of DNA makes RNA makes protein, although the book does include a good deal of the newer eukaryote material. A glance at almost any topic shows how this gutsy discipline gets watered down in texts which try to assimilate it with the whole of the new biology. I wonder if this particular subset of the information will continue to be sustainable, however, as the tools of the trade are applied increasingly to supracellular problems such as development. A hint of future difficulties can be felt in Freifelder's need to sketch in matters cell biological in order to discuss the action of oncogenes.

Schleif's *Genetics & Molecular Biology* covers much of the same ground, but otherwise is a world apart, and a remarkable achievement. It is an advanced text, derived from material taught largely to postgraduates, and will probably be thought best suited to budding professionals in molecular genetics. In some ways this would be a pity, because there is also gold here for the rest of us. One feature is the prominence given to thinking about the engineering logic of the systems under consideration. For example, why are great complexities engendered by discontinuous replication of DNA on the lagging strand when cells could perfectly well have evolved a polymerase working continuously 3' to 5'? How do bacteria survive the probable error rates of their own restriction enzymes? The answers are perhaps given rather dogmatically, but other authors do not pose such questions let alone open discussion of subjects with them.

A second feature of Schleif's book is the constant encouragement of calculations, both in the text and in the challenging problems, reminding us that molecular biologists should still keep a stock of old envelopes as well as restriction enzymes. "Deeper Reading" lists which follow each chapter mean just that — in some areas they appear to cover an appreciable proportion of the primary literature. The lessons here in dealing with the information explosion in biology are that an ounce of rationale is worth a pound of facts and that, for educational value, there is nothing to beat an author writing about stuff he knows from the inside. □

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